Notes on the pubescence of *Zornia bracteata* with description of a new form

John Michael Kelley

Kelley Botanical Library; Haughton, Louisiana 71037, USA
kelley.johnmichael@gmail.com

ABSTRACT

Previous descriptions of *Zornia bracteata* have inadequately and incompletely described the indument of the species. After a rangewide examination, forma *strigillosa* is described for populations on the pubescent extreme. *Published online* ***www.phytologia.org*** *Phytologia 106(4): 47-50 (December 20, 2024). ISSN 030319430.*

KEY WORDS: Forma, Texas, Alabama

Zornia bracteata Walt. ex Gmel. (Fabaceae) is a low, reclinate-sprawling herb with peltate-compound leaves, each with four leaflets, bearing yellow flowers in late spring and summer, each enclosed in a pair of foliaceous bracts, and bearing spiny loment fruits in late-summer and fall (Mohlenbrock 2023; Small 1933; pers. obs.). The species is restricted to xeric sands in the coastal plain states (Weakley 2022). While some literature has described morphological aspects of the species in detail (Mohlenbrock 2023; Small 1933), aspects of the pubescence in particular have been poorly described in prior treatments, with the plant sometimes described as glabrous (Britton and Brown 1913; Correll and Johnston 1970; Gleason and Cronquist 1963; Diggs et al. 1999; Gleason 1952; Mohlenbrock 1961; Radford et al. 1968).

Notice of undescribed variation in the indument of Louisiana plants inspired me to investigate the pubescence of the species rangewide. To do so, I examined all occurrences on iNaturalist (https://www.inaturalist.org/), many on the Symbiota portals (https://symbiota.org/symbiota-portals/), and all specimens in the BRIT, VDB, and NLU collections. I paid particular attention to trichomes on the stem, petioles, petiolules, leaf laminae, bract faces and margins, calyx, and fruits. In the 300-400 samples/observations I examined, I found the following conditions:

The plants are variable across their geographical range, with leaf shape and plant stature varying within populations. Pubescence is less variable within populations, but certainly varies greatly over short distances. Thus, a plant in one Alabama county may be densely strigillose, while those in the adjacent county are less so, and those a few counties farther are essentially glabrous. Intergrading forms abound, and the extremes of indument are each relatively rare and localized.

The petiolules of the leaflets are almost invariably hairy, often canescent with stiff white hairs. The margins of the floral bracts too are rarely glabrous, but they range from less than a dozen cilia along the margin to more than one hundred, mostly in the distal half. The petioles and stems are ascendingly strigillose a bit less often, and the leaf margins ciliate less often still. The abaxial midribs are often strigillose, more rarely the adaxial surface is pubescent too, and very rarely the two faces are conspicuously canescent hairy. Associated with the pubescence of the leaf, and among the rarest states, is a densely strigillose condition on the faces of the floral bracts. The fruits are covered in retrorsely barbed spines, and the barbs can become almost hairlike or be entirely absent at their extremes. The interstitial space between the spines varies from glabrous to densely and stiffly puberulent. The faces are reticulated.

Across most of the range, the plants are glabrate overall, and they are in fact glabrous in some Florida populations. Scattered populations, however, embody the opposite extreme; plants from the Coastal Bend of Texas and southeast Alabama are the most densely and thoroughly pubescent, exhibiting all the densest conditions described above.

In light of this examination and being justified by the dearth of accurate description in prior works, I have decided to name a form for these populations. The type specimen is one of a handful of specimens from the coastal bend which Dr. Billie Turner† annotated only to genus. This is unusual in the body of his collections and suggests that he could not confidently ascribe this form to a species; apparently, he never finished an investigation of the genus. No valid subspecific taxa exist which accommodate the characters described (Tropicos, https://www.tropicos.org/).

Zornia bracteata Walt. ex Gmel. forma ***strigillosa*** J.M. Kelley **f. nov. - TYPE: USA. Texas.** Aransas Co.: St. Joseph Island, 10 miles east of Rockport, low dunes and mudflats, 21 April 1965, *B.L.Turner* 5177 (holotype: BRIT).

DIAGNOSIS

Similar to the typical form, except with moderate to dense pubescence on multiple parts, such as: strigillose pubescence on the stem (especially the upper sections), petioles, and abaxial faces of the leaf; fine pubescence on the abaxial (and often adaxial) surfaces of the floral bracts and dense cilia along their margins; fruits with the lower barbs on the spines canescent and somewhat hairlike and with sparse to dense hairs in the interstitial space between the spines (Figs. 1, 2). This form is frequently expressed in southeast Alabama and predominates in populations along the Coastal Bend of Texas.

ACKNOWLEDGEMENTS

I thank my reviewers, Jason Singhurst (Texas Parks and Wildlife Dept.) and Eric Keith (Raven Environmental Services, Inc.). Robert Mohlenbrock provided encouragement in the investigation and his FNA treatment was closest to the reality of this species. Edwin Bridges kindly offered his perspective on Dr. Turner's intent. Ashley Bordelon (BRIT) made herbarium work painless, and Jacob Hamsher helped in sorting specimens.

LITERATURE CITED

Britton, N. L. and A. Brown. 1913. An Illustrated Flora of Northern United States and Canada. 1st ed. 3 vols. Charles Scribner's Sons, New York.

Correll. D. S. and M. C. Johnston. 1970. Manual of the vascular plants of Texas. Texas Research Foundation, Renner, Texas.

Diggs, G. M., B. L. Lipscomb, and R. J. O'Kennon. 1999. Illustrated Flora of North Central Texas. Sida Bot. Misc. 16.

Gleason, H. A. 1952. The New Britton and Brown Illustrated Flora of the Northeastern United States and Adjacent Canada. The New York Botanical Garden, Bronx, New York.

Gleason, H. A. and A. Cronquist. 1963. Manual of Vascular Plants of Northeastern United States and Adjacent Canada. D. Van Nostrand Company, New York.

Mohlenbrock, R. 1961. A monograph of the leguminous genus Zornia. Webbia 16: 1–141.

Mohlenbrock, R. 2023. Zornia. In Flora of North America Committee (eds.). Flora of North America North of Mexico. Volume 11, Magnoliophyta: Solanales. Oxford Univ. Press, New York and Oxford, UK.

Radford, A. E., H. E. Ahles, and C. R. Bell. 1968. Manual of the vascular flora of the Carolinas. Univ. of North Carolina Press, Chapel Hill, North Carolina.

SERNEC Data Portal. 2020. (http//:sernecportal.org/portal/index.php, Accessed March 2022).

Small, J. K. 1933. Manual of the Southeastern Flora. Univ. of North Carolina Press, Chapel Hill.

Weakley, A. S., and Southeastern Flora Team 2022. Flora of the southeastern United States. University of North Carolina Herbarium, North Carolina Botanical Garden.

Figure 1. Forma *strigillosa* expressions from Texas with ciliate leaf margins (left, Brian Finzel CC-BY), strigillose upper stems with bracts ciliate and pubescent on both faces (upper right, Brian Finzel CC-BY), and the pubescent fruits with canescent hairs in the interstitial space below the spines (bottom right, Cleveland Powell CC-BY).

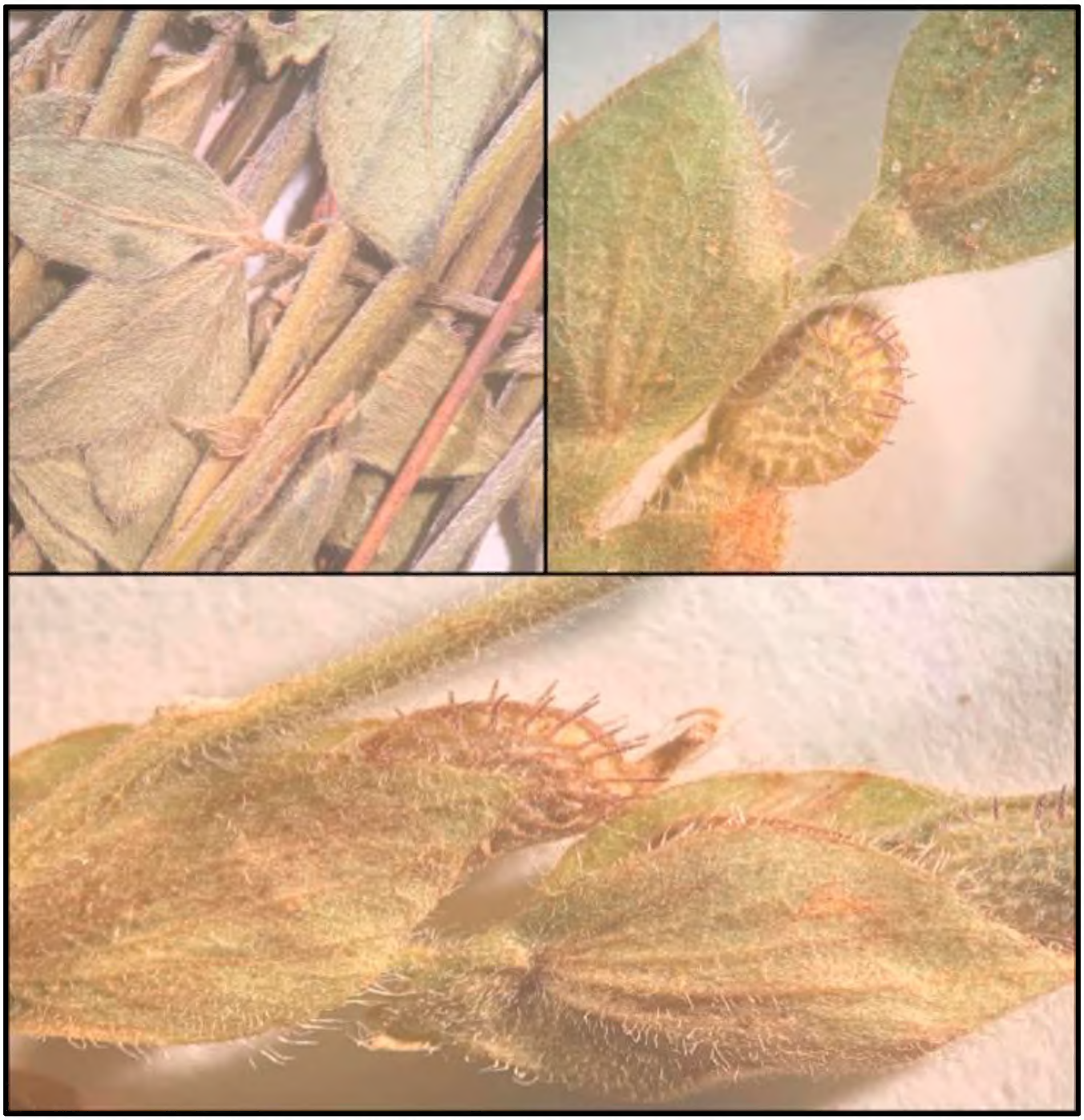


Figure 2. Forma *strigillosa* expressions from Barbour and the surrounding counties in Alabama.

Sommerstorffia pugioniformis sp. nov: A new species of rotifer-trapping oomycete discovered using a novel baiting technique

Paul G. Davison
Department of Biology, University of North Alabama, Florence, AL 35632, USA, pgdavison@una.edu

Jeffery M. Ray
Department of Biology, University of North Alabama, Florence, AL 35632, USA, jmray1@una.edu

Carrie Ann Davison
Department of Genetics, Yale University, New Haven, CT 06510, USA, carrieann.davison@yale.edu

ABSTRACT

The genus *Sommerstorffia* (Oomycota, Saprolegniales, Verrucalvaceae) is a predator of freshwater rotifers last recorded in North America sixty years ago. Here we describe a novel baiting technique for *Sommerstorffia* that relies on the easily cultured rotifer *Lecane inermis* and plastic coverslips as a transparent substrate for *Sommerstorffia*'s attachment. The efficacy of the baiting technique is supported by our multiple records of *Sommerstorffia spinosa* across seven U.S. states. With this technique, we discovered sporeling morphology quite different from *S. spinosa* leading us to propose *Sommerstorffia pugioniformis* as a new species. Molecular analysis based on COI and ITS regions placed the new species and *S. spinosa* within the Verrucalvaceae clade. *Published online* ***www.phytologia.org*** *Phytologia 106(4): 51-67 (December 20, 2024). ISSN 030319430.*

KEY WORDS: *Lecane inermis*, *Sommerstorffia pugioniformis*, *S. spinosa*, baiting, oomycete, predatory fungi, standing drop, rotifers, Verrucalvaceae.

Several lineages of fungi and fungal-like organisms are known to parasitize or prey on rotifers (Barron 2004; Glockling et al. 2014). Within the monophyletic Verrucalvaceae (Oomycota, Saprolegniales) three genera include species parasitic/predatory on rotifers. The taxonomically diverse genus *Aphanomyces*, best known for several economically important species parasitic on plants and animals (Becking et al. 2022), includes two questionable species, *A. americanus* and *A. hydatinae*, that are parasitic on rotifers but are not known beyond their original descriptions (Scott 1961; Molloy et al. 2014). More recently, a new genus *Aquastella* was described to accommodate two species that are obligate rotifer parasites, *A. acicularis* and *A. attenuata*, known only from planktonic rotifer hosts collected over a seven-year period in Brooktrout Lake, Adirondack Park, New York State (Molloy et al. 2014). Finally, *Sommerstorffia*, with only *S. spinosa* Arnautov (1923a), is a globally widespread predator of rotifers.

Sommerstorffia spinosa is a predator of common, periphytic, loricate rotifers in freshwater habitats (Glockling et al. 2014). *Sommerstorffia*'s structure and development was described in detail by Karling (1952) who made a prolonged study of a culture derived from an Alaskan alpine tundra stream. Even to a nonspecialist, *Sommerstorffia* is quite recognizable based on the distinctive stiff hyphal branches that end in a predaceous peg. Sporelings that form from encysted zoospores also bear a predaceous peg. Rotifers grazing for food bite the predaceous pegs and thereby become trapped by the mouth by an adhesive substance (Karling 1952; Saikawa and Hoshino 1986; Saikawa et al. 2021). A lobed zoosporangium forms inside the body of the rotifer, and zoospores are released through an exit tube in achlyoid fashion.

The published discoveries of *Sommerstorffia spinosa* appear to have been made without prediction and were typically made weeks (Karling 1952; Saikawa and Hoshino 1986) or months (Sparrow 1929; Prowse 1954; Czeczuga et al. 2000) after initiation of gross cultures usually augmented with traditional baits (e.g., onion skin, snake skin, plant seeds) for the recovery of various saprobic water molds.

Presumably these lengthy intervals between culture initiation and *Sommerstorffia* detection allowed host rotifer populations to increase and thereby favor *Sommerstorffia*'s rise to detectable levels. The inconspicuous nature of *S. spinosa* and its dependence on rotifers susceptible to the trapping mechanism may be among the reasons few freshwater mycologists have reported *Sommerstorffia* in published field studies of oomycetes. To our knowledge, the last known published observation from North America was made in 1963 by Charles E. Miller from Mountain Lake Biological Station, Virginia (Miller 1965).

As a result of collecting samples for an undergraduate botany course, P. G. Davison observed *Sommerstorffia spinosa* from two springs in north Alabama in 2011 and 2013. Since 2013, P. G. Davison developed a baiting technique that allows for relatively rapid lab-based detection of *Sommerstorffia* in field samples. The baiting technique relies on the easily cultured rotifer *Lecane inermis* (Bryce, 1892) as bait. We applied this method to a sample from Mill River, Connecticut, which led to the discovery of *Sommerstorffia* nearly identical to *S. spinosa* except for the unique shape of its sporelings. We describe the novel baiting technique and our method of culture from single spore isolates below along with a description of the proposed new species *Sommerstorffia pugioniformis*. Our observations provide the first documented occurrences of *S. spinosa* in Alabama, Connecticut, Florida, Mississippi, North Carolina, and Tennessee.

In 2013 Alabama material of *S. spinosa* fixed in RNAlater was sent to Christoffel F.J. Spies and C. André Lévesque who isolated the LSU and SSU regions (unpublished, see Glockling et al. 2014; Molloy et al. 2014) that indicated *Sommerstorffia* was closely related to *Aphanomyces* as had been previously suggested by Arnaudov (1923a) and Karling (1952). Based on trustworthy, though unpublished, sequence data *Sommerstorffia*'s placement in the Verrucalvaceae was accepted (Beakes and Thines 2017, Rocha et al. 2018). We generated and analyzed Cytochrome c oxidase subunit I (COI) and the internal transcribed spacer (ITS) recommended by Robideau et al. (2011) as the complementary DNA barcodes used for taxonomic identification of oomycetes. With this analysis, we provide the first published sequences supporting *Sommerstorffia*'s placement in the Verrucalvaceae. Additionally, we provide sequence data in support of recognizing two species of *Sommerstorffia*, *S. spinosa* and *S. pugioniformis*.

MATERIALS AND METHODS

Baiting technique. Field samples of submerged substrates (plant matter, sediment, debris), surface scum, and source water were collected in sterile Whirl-Pak® bags. Samples were refrigerated if not treated the same day. Within three days, collected material was transferred by sterile tweezers and/or pipette to a 6 cm diameter Petri dish or a 6 cm square dish containing a few ml of bottled FIJI® Water (Fig. 1). Next, several thousand *Lecane inermis* in 3 mL of rotifer culture and a floating plastic coverslip (Fisherbrand™ disposable cover slips, 22 × 22 mm) as potential substrate for *Sommerstorffia*, were added to each dish. Coverslips were monitored *in situ* for up to seven days with a compound microscope (Fig. 2).

The bait rotifer *Lecane inermis* was isolated in 2013 from a spring in Florence, Alabama. Derived from a clone of a single individual washed through several drops of bottled spring water, *Lecane inermis* was cultured in 10 cm diameter Petri dishes containing 25 ml of FIJI Water and three rice grains (Mahatma basmati rice) or 0.25 ml of V-8 Juice. Rotifers consumed unidentified bacteria and particulate debris in the cultures. Subcultures were made at least every three weeks by inoculating sterile dishes with 3 ml of an existing culture. Segars (1994-95) was used to identify the rotifer species.

Producing single spore isolates. Coverslips bearing *Sommerstorffia* were placed specimen side up on a glass pedestal (Figs. 3-4), and several drops of rotifer culture were added forming a modified sitting drop culture that is here called a standing pool culture. After three days in a moist chamber the standing pool was rinsed with distilled water in an attempt to eliminate infusoria and a few drops of rotifer culture were added. After 8-12 hours the standing pool was rinsed again to remove uncaptured rotifers. The standing pool was monitored through a compound microscope over several days for the development of mature sporelings in the surface film. It was sometimes necessary to repeat the process of adding rotifers followed by rinsing to acquire numerous sporelings in the pool's surface. A few drops of rotifer culture were then added and the surface immediately monitoring through a dissecting microscope for sporeling-trapped

rotifers (detectable by the rotifer's body writhing while the rotifer's head remained stationary). Within 15 minutes of being trapped, rotifers were picked up with a flame sterilized Irwin loop (Schram and Davison 2012) and washed through several separate drops of FIJI Water. The final transfer was to a sitting drop of FIJI Water (Fig. 5) to which several drops of rotifer culture were then added. This sitting drop culture was held in a moist chamber and monitored for up to five days. Sitting drop cultures with mature hyphae and/or sporelings were transferred as floating coverslips to individual 10 cm diameter Petri dishes freshly prepared with three rice grains, 3 ml of rotifer culture, and 25 ml of FIJI Water.

Maintenance of *Sommerstorffia* cultures. Cultures derived from single spore isolates were kept at room conditions and subcultured every 30 days as follows. *Sommerstorffia*-infected plastic coverslips (or fragments of plastic coverslips) bearing hyphal pegs and/or sporelings were transferred to freshly prepared Petri dishes containing three rice grains, 3 ml of rotifer culture, and 25 ml of FIJI Water.

Photography and Measurements. Micrographs were taken through a Nikon Alphaphot-2 YS2 or an Eclipse E200 compound microscope, fitted with a Handycam HDV 1080i camera with an adaptor from Martin Microscope Company or an iPhone 14 Pro with LabCam® adaptor from iDu Optics®. Measurements were made from water-mounted *Sommerstorffia* in the living state. Imaged material was attached to plastic or glass coverslips floated in Petri dish cultures (slide mounted for photomicroscopy). Glass coverslips were held in the surface water of cultures by a plastic bracket that prevented the glass from sinking (Fig. 6). Measurements were made from digital photographs in ImageJ (https://imagej.net/ij/) calibrated by images of a stage micrometer taken at same scale. Distracting background artifacts were removed with Adobe Photoshop in some images.

Preservation of dried voucher specimens. Plastic coverslips bearing *Sommerstorffia* in various stages of development were dried specimen side up on filter paper in a vented Petri dish. Following Wu et al. (2004), Petri dishes were placed in archival quality paper packets with attached labels. Specimens were deposited in the Kriebel Herbarium (PUL) at Purdue University, the Yale University Herbarium (YU), and the University of North Alabama Herbarium (UNAF).

Molecular Methods. Samples for DNA extraction were processed from Petri dish cultures, which were decanted by pipetting, leaving surface sporelings and thalli in the dish with minimal water. A sterile razor blade was used to scrape the dish and concentrate material to one side. Approximately 1 ml volume was pipetted into a 1.7 ml sterile tube and centrifuged at 10,000 rpm for 3 min, after which a pellet was visible in the bottom of the tube. The supernatant was removed, and the tube with pellet was placed in an ultralow freezer at -80°C. After 5 min, the frozen pellet was ground by hand using a disposable plastic pestle to mechanically disrupt the cells. The 1.7 ml tube was then utilized for the first steps of DNA extraction using a Qiagen DNeasy® Plant Mini Kit (catalog no. 69104), following the Quick Start Protocol (QIAGEN).

PCR reactions used a total volume of 50 uL including 4 uL template DNA, 25uL Promega GoTaq® Green Master Mix (catalog no. M7122), 2.5uL each primer (0.3uM final concentration), and 16 uL nuclease-free water. For COI, primer pairs were OomCoxI-Levup – OomCoxI-Levlo and OomCoxI-Levup – FM85mod (Robideau et al. 2011). For ITS, primer pairs were ITS1oo–ITS4(r) and ITS3oo–ITS4(r) (White et al. 1990; Ritt et al. 2016, 2018). A Techne ^{3}Prime G Thermo Cycler (Bibby Scientific) was used for the following conditions: (1) an initial denaturation of 60 s at 94°C, (2) 35 cycles of 60 s at 95°C, 30 s at 55°C, 60 s at 72°C, and (3) a final extension of 60 s at 72°C. PCR products were verified for appropriately-sized bands on a 2% agarose gel stained with EtBr; electrophoresis was for 40 min at 70 W. Products were shipped to Eurofins Genomics, Louisville, KY, USA for PCR purification and Sanger sequencing of both forward and reverse strands using the identical forward and reverse PCR primers listed above.

Specimens with data for both COI and ITS markers from Verrucalvaceae and Saprolegniaceae, along with outgroup taxa *Apodachlya brachynema* and *A. minima* (Robideau et al. 2011; Rocha et al. 2018),

were downloaded from GenBank. Sequencing results were edited and aligned with GenBank taxa in Geneious Prime 2023.2.1 producing an alignment of 19 sequences with 1,583 characters (680 for COI and 903 for ITS). New *Sommerstorffia* DNA sequences were deposited in GenBank, with accession numbers PP404027–PP404033 for COI and PP407290–PP407296 for ITS (Table 1). The software ModelFinder (Kalyaanamoorthy et al. 2017) was used to determine the optimum model of nucleotide substitution for each genetic marker and performed a maximum likelihood (ML) analysis on the partitioned dataset in IQ-TREE (Trifinopoulos et al. 2016). Settings for the best-fit substitution model were TPM2+F+G4 (COI) and TVM+F+I+G4 (ITS), with ultrafast bootstrap support analysis using 1000 alignments (Hoang et al. 2018). The resulting ML tree was modified for viewing with iTOL (Letunic and Bork 2021).

Table 1. Taxa included in phylogenetic analyses including reference/locality, isolate, and GenBank accession numbers for specimens. Data downloaded from GenBank includes taxa from Robideau et al. (2011). Taxa and isolates correspond to specimens in Figure 7.

Oomycete Taxa	Reference / Locality (State: County)	Isolate	GenBank Accession Number	
			COI	ITS
Apodachlya brachynema	Robideau et al. 2011	CBS 184.82	HQ708197	HQ643124
Apodachlya minima	Robideau et al. 2011	CBS 185.82	HQ708199	HQ643126
Pythiopsis terrestris	Robideau et al. 2011	CBS 110059	HQ708453	HQ643406
Achlya papillosa	Robideau et al. 2011	CBS 101.52	HQ708175	HQ643102
Achlya spinosa	Robideau et al. 2011	CBS 576.67	HQ708182	HQ643109
Leptolegnia caudata	Robideau et al. 2011	CBS 680.69	HQ708211	HQ643137
Protoachlya paradoxa	Robideau et al. 2011	CBS 100.44	HQ708451	HQ643404
Saprolegnia ferax	Robideau et al. 2011	BR 114	HQ709029	HQ643988
Saprolegnia parasitica	Robideau et al. 2011	CBS 113187	HQ709046	HQ644005
Plectospira myriandra	Robideau et al. 2011	CBS 523.87	HQ708449	HQ643402
Aphanomyces cochlioides	Robideau et al. 2011	CBS 477.71	HQ708188	HQ643115
Aphanomyces cladogamus	Robideau et al. 2011	CBS 108.29	HQ708186	HQ643113
Sommerstorffia pugioniformis sp. nov.	CT: New Haven	CT03	PP404028	PP407291
Sommerstorffia pugioniformis sp. nov.	CT: New Haven	CT04	PP404029	PP407292
Sommerstorffia spinosa	CT: New Haven	CT01	PP404027	PP407290
Sommerstorffia spinosa	FL: Taylor	SP05	PP404030	PP407293
Sommerstorffia spinosa	FL: Sarasota	SP06	PP404031	PP407294
Sommerstorffia spinosa	AL: Lawrence	SP07	PP404032	PP407295
Sommerstorffia spinosa	AL: Lauderdale	SP08	PP404033	PP407296

RESULTS

Phylogenetic analysis. The final alignment contained 464 parsimony-informative characters (COI: 122 characters, ITS: 342 characters). Maximum Likelihood analysis recovered all *Sommerstorffia* samples in a well-supported clade (100% bootstrap value), sister to two other genera within Verrucalvaceae, and more distantly related to species recognized as members of Saprolegniaceae (Fig. 7). *Sommerstorffia* sp. nov. in Connecticut, USA was recovered as a well-supported clade divergent from the sympatric (and syntopic) *S. spinosa* in Connecticut. The widely disjunct (geographic distances of ca. 1,700-2,000 km) samples of *S. spinosa* from Connecticut, Alabama, and Florida formed a well-supported clade. Uncorrected sequence divergence between *Sommerstorffia* species was 3.5–2.7%, with intraspecific divergences of 0.4% (*S. pugioniformis sp.* nov.) and 0.4–1.6% (*S. spinosa*).

TAXONOMY

Sommerstorffia pugioniformis P.G. Davison, J.M. Ray, and C.A. Davison, sp. nov. Figs. 8 – 21, 27.
MycoBank no.: MB 855653

Typification: USA, Connecticut, New Haven County, New Haven, East Rock Park, 41.33292°N, 72.90932°W, 0.6 m, from a sample of water with submerged vegetation and sediment collected along margin of Mill River by P.G. and C.A. Davison, June 6, 2020; strain CT03 isolated by P.G. Davison. HOLOTYPE Fig. 8, this publication.

Etymology: The specific epithet refers to the sporeling being dagger like; from the Latin words *pugio* = dagger and *forma* = shape.

Diagnosis: Similar to *Sommerstorffia spinosa*, however, *Sommerstorffia pugioniformis* differs from *S. spinosa* as follows: the neck of the sporeling is 1.2–2 times longer than the length of the base of the sporeling (versus the neck about half the length of the base); the neck of the sporeling tapers ending in a minute subglobose apex (versus neck not tapered, ending in a blunt tip). Additionally, the hyphal pegs of *S. pugioniformis* are more narrowly pointed, being 1 µm wide (versus 2–5 µm wide).

Description: Thallus external to host rotifer consisting of tubular hyphae, (1–)2–5(–6) branched, branches becoming more numerous with subsequent rotifer captures; hyphal branches 35–76 µm long × 3.3–5.0 µm wide; base of branches sometimes contorted in zig-zag fashion, region where two or more branches originate sometimes swollen to 7–14 µm wide; distal ends of branches sweeping away from substrate; branch tips ending in a predaceous peg 3.0–5.2 µm long × 0.9–1.2 µm wide, often with a thin extrusion of unknown material extending 2–4 µm beyond the apex, pegs sometime slightly swollen at the apex. Thallus inside host rotifer a lobed zoosporangium filling wholly, or in part, the rotifer body, the lobes up to 17 µm in diameter. Exit tubes 14–70 µm long × 4–6 µm wide. Cystospores 6–9 µm diameter, numbering 12–60 atop the exit tube. Zoospores laterally biflagellate, reniform, 6.0–8.6 × 5.2–6.8 µm (measured in rocking phase atop exit tube). Sporelings consisting of a swollen base and a neck that serves as a predaceous peg; the base lemon-shaped to subglobose, 6.8–10 µm long × 5.1–7.5 µm wide, often with a narrow, transverse, irregular band of clear cytoplasm; the neck 11.1–17.3(–21.7) µm long × 2.0–2.7 µm wide, tapering in the distal ¼–½ to a short, narrow isthmus 0.2–0.6 µm wide tipped by a subglobose head 0.7–1.1 µm in diameter. Zoospores encysting twice, once atop the exit tube of zoosporangium and again after flagellated dispersal; second cyst attached to submerged surfaces or to the thin film at the water's surface. Second cyst releases a sporeling that remains attached to substrate. Oogonia terminating 12–74 µm long hyphal branches, bullate, 23–30 µm outer diameter. Oospheres single, usually not maturing, 17–20 µm diameter. Antheridia not seen.

Geographical distribution: **North America**, USA, Connecticut, New Haven.
Habitat: Known only from shallow, bankside water in the Mill River downstream of the Lake Whitney dam. See Fig. 29 for photograph of the type locality.

Host selection: *cf. Lepadella*, *Lecane inermis*.

Remarks: Development of oogonia was sporadic and restricted to older cultures. Almost all development can be classified as endo-epibiotic as described by Karling (1952) in which both an internal zoosporangium and external hyphae bearing pegs form from a sporeling that has captured a rotifer. Rarely, as noted by Karling for *S. spinosa*, development is completely endobiotic (internal zoosporangium but no external hyphae) (Fig. 18) or epibiotic (external hyphae form but no zoosporangium).

As in *S. spinosa*, there is often an extension of hyaline material (Fig. 10) that protrudes beyond the predaceous pegs. Arnaudov and Damova (1947-1948) first noticed these extensions and questioned their purpose. Karling (1952) stated the material was not adhesive to passing rotifers and the purpose of the extensions was unknown. Prowse (1954), unconvincingly, concluded the material was mucilage that appeared to capture rotifers. Having observed the grazing habits of *Lecane inermis* actively chewing and completely consuming in cannibalistic fashion the ghostly remains of partially decomposed rotifers and membranous material in V-8 juice, a plausible explanation for these extensions is that they serve to stimulate the grazing activity of the rotifer leading it to the predaceous peg. Only by biting the peg is the rotifer trapped. We hypothesize that the hyaline extensions are an adaptation that improves the efficacy of the hyphal pegs in trapping rotifers.

Additional specimens examined: **USA. Connecticut**: New Haven County, New Haven, Mill River, East Rock Park, 41.33292°N, 72.90932°W, 0.6 m, submerged vegetation and sediment, 7 Jun 2021, P.G. and C.A. Davison, occurring sympatrically with *S. spinosa*, no specimen preserved; same data except 41.32614°N, W 72.90701°W, 0.6 m, patch of surface scum (few cm in diameter) 0.5 m from shore, preying on native *cf. Lepadella*, 7 Jun 2021, P.G. and C.A. Davison 2021-06-07.2 (PUL, UNAF, YU)

Sommerstorffia spinosa Arnautov, Flora (Regensburg) 116: 109 (1923a), Figs. 22 – 26, 28
Lectotype designated by Dick (2001), Flora, Jena 116: 109-113, figs 1-5 (Arnaudov 1923a)

Description: see Karling (1952, 1966).

Geographical distribution: ASIA. Japan (Saikawa and Hoshino 1986; Saikawa et al. 2021), India (Karling 1966) AUSTRALASIA. New Zealand (Karling 1968), EUROPE. Bulgaria (Arnaudov 1923a,b), Czechoslovakia (Cejp 1959 *fide* citation in Johnson et al. 2002); England (Prowse 1954), Poland (Próba 1980, Czeczuga and Próba 1980; Czeczuga et al. 2015); Russia (https://www.inaturalist.org/observations/163541892). NORTH AMERICA. USA. Alaska, Louisiana, New Jersey (Karling 1952), Virginia (Karling 1952; Miller 1965), Massachusetts (Sparrow 1929), New York (Sparrow 1933; this study), Alabama, Florida, Mississippi, North Carolina, Tennessee (this study), West Indies, Cuba (Sparrow 1952).

Habitat: Freshwater ponds, lakes, springs, rivers, soil (Arnaudov 1923a; Czeczuga et al. 2000; Karling 1952, 1966, 1968; Próba 1980; Saikawa and Hoshino 1986). See Fig. 30 for photograph of the type locality. Native substrates as reported in the literature (substrates used as bait not included): *Cladophora* (Arnaudov 1923a, Prowse 1954, Sparrow 1933), *Rhizoclonium* (Sparrow 1929), littoral detritus (Saikawa and Hoshino 1986), soil—presumably aquatic sediment—(Karling 1952, 1966, 1968). Native substrates with attached thalli observed in baited dishes (this study): *Ceratophyllum demersum*, *Chara* sp., *Fissidens fontanus*, *Najas guadalupensis*, *Podostemum ceratophyllum*, *Spirodela polyrhiza*, *Utricularia* sp.

Host selection: Rotifers: *Lecane* sp., *Lecane inermis* (this study), *Colurella* sp. and *Lepadella* sp. (Glocking et al. 2014); protists: *Entosiphon ovatum* (Karling 1952), *Difflugia pyriformis* (Saikawa et al. 2021).

Remarks: So far, oogonia in *S. spinosa* have only been seen in European material (Arnautov 1923a; Czeczuga and Próba 1980; Próba 1980; Czeczuga et al. 2000). Karling (1952) did not observe oogonia in the Alaskan material he maintained in culture for over a year. Likewise, oogonia have not been seen in any of our isolates from seven U.S. states maintained in culture for 1–4 years.

Additional specimens examined: **USA**. **Alabama**: Colbert County, Cane Creek boat ramp, 34.75422°N, 87.86181°W, 126 m, *Spirodela polyrhiza* floating between cattail stalks, 7 Aug 2020, P.G. Davison, no specimen preserved; Tennessee River just upstream of confluence with Cane Creek, 34.75554°N, 87.86135°W, 126 m, *Najas guadalupensis* submerged near shoreline, 7 Aug 2020, P.G. Davison, no specimen preserved; Sheffield, Tennessee River, 34.76739°N, 87.71145°W, 126 m, algae attached to underpinning of floating dock, 12 Aug 2020, P.G. Davison, no specimen preserved; Lauderdale County, Florence, King Spring, 34.85683°N, 87.65412°W, 165 m, submerged dialysis tubing, Feb 2011, P.G. Davison, no specimen preserved; Big Spring north of Hwy 20, 34.78945°N, 87.68301°W, 128 m, duckweed community baited with poppy seeds, Jan 2013, P.G. Davison, preying on *Lecane inermis*, no specimen preserved; unnamed stream on the campus of the University of North Alabama, submerged *Fissidens fontanus*, 34.80643°N, 87.68607°W, 150 m, 24 Dec 2020, P.G. Davison 2020-12-24.1 (PUL, UNAF, YU); Wildwood Park, Cypress Creek, 34.80370°N, 87.69524°W, 128 m, lower stems of *Podostemum ceratophyllum*, 3 Nov 2023, P.G. Davison 2023-11-03.1 (PUL, UNAF, YU); Wildwood Park, upland headwater spring under forest cover, 34.81113°N, 87.69456°W, 150 m, submerged brown detritus, 8 Jul 2023, P.G. Davison, no specimen preserved; McFarland Park, floodplain of Tennessee River, golf course pond, 34.78670°N, 87.68186°W, 127 m, *Spirodela polyrhiza* floating on pond, 5 Jul 2023, P.G. Davison, no specimen preserved; McFarland Park, slough along Hwy 20, 34.78805°N, 87.68555°W, 127 m, *Lemna* sp. and *Landoltia punctata* floating in surface, 5 Jul 2023, P.G. Davison, no specimen preserved; Lawrence County, Prairie Grove Glade Preserve, 34.51724°N, 87.50322°W, 180 m, *Chara* in small pond, 24 Mar 2021, P.G. Davison 2021-03-24.1 (PUL, UNAF, YU); **Connecticut**: New Haven County, New Haven, Mill River, East Rock Park, 41.33292°N, 72.90932°W, 0.6 m, submerged vegetation and sediment, 7 June 2021, P.G. and C.A. Davison 2021-06-07.1 (PUL, UNAF, YU); Regional Water Authority Recreational Area, 41.33579°N, 72.91281°W, 1 m, *Spirodela polyrhiza* floating on pond, 6 Jun 2020, P.G. and C.A. Davison, no specimen preserved; **Florida**: Taylor County, Steinhatchee River, 29.67580°N, 83.36589°W, 0.5 m, floating and submerged woody debris, 29 Jul 2021, P.G. Davison 2021-07-29.1 (PUL, UNAF, YU); Sarasota County, Lake Jervey, State College of Florida Venice campus, 27.04335°N, 82.31958°W, 1 m, submerged debris, 26 Dec 2020, E.L. Kasl 2020-12-26.1 (PUL, UNAF, YU); Sarasota Golf Community, roadside ponds, 27.03914°N, 82.38327°W, and 27.03635°N, 82.36362°W, 4 m, submerged debris, 26 Dec 2020, E.L. Kasl, no specimens preserved; **Mississippi**: Tishomingo County, Tishomingo State Park, Haynes Lake, 34.61559°N, 88.19349°W, 145 m, *Chara* and sediment, 17 Jul 2023, P.G. Davison 2023-07-17.1 (PUL, UNAF, YU); **New York**: Putnam County, Town of Kent, Veterans Memorial Park, 41.44194°N, 73.70960°W, 177 m, *Ceratophyllum*, *Potamogeton*, and debris, 11 Jun 2022, P.G. Davison 2022-06-11.1 (PUL, UNAF, YU). **North Carolina**: Gaston County, Crowders Mountain State Park, Shorts Lake, N 35.20761°N, W 81.29243°W, 252 m, *Utricularia* and sediment, 16 Mar 2024, P.G. Davison 2024-03-16.1 (PUL, UNAF, YU); Watauga County, Blowing Rock, Mayview Lake, 36.13370°N, 81.68141°W, 1074 m, surface scum and submerged *Callitriche*, 28 Jul 2022, P.G. Davison 2022-07-28.1 (PUL, UNAF, YU); **Tennessee**: Hawkins County, Holston River at Surgoinsville, 36.46853°N, 82.85095°W, 333 m, submerged leaves of *Vallisneria americana*, 18 Oct 2023, D.H. Webb, no specimen preserved; Lawrence County: Holly Creek near confluence with Shoal Creek, 35.02527°N, 87.57945°W, 165 m, exposed, submerged roots of *Justicia americana*, 1 Aug 2023, P.G. Davison and J.M. Ray 2023-08-01.1 (PUL, UNAF, YU).

DISCUSSION

Based on both morphological and phylogenetic analyses, *S. pugioniformis* is described as distinct species within a previously monotypic genus. The phylogenetic position of *Sommerstorffia* within the Verrucalvaceae (Beakes and Thines 2017; Rocha et al. 2018) is confirmed using data from the two recommended genetic markers (Robideau et al. 2011).

When detectable, *Sommerstorffia* was found attached to floating coverslips 3 to 6 days after the addition of bait rotifers. As early as one day after baiting, rotifers may be seen head planted on the surface of opaque, submerged material where it is difficult to see evidence of a specific predator. The rotifer's body projecting away from the substrate may be seen in profile. Such instances are often indicative of the rotifer having been trapped by either *Sommerstorffia* or the zygomycete fungus *Zoophagus*. From our observations and others (Arnaudov 1923b; Sparrow 1933; Saikawa et al. 2021) *Sommerstorffia spinosa* and *Zoophagus cf. insidians* frequently co-occur. Either predator may appear on the dish bottom or attached to submerged plants or debris. Only *Sommerstorrfia* readily spreads by zoospores and soon appears with trapped rotifers under the transparent coverslip where its distinctive morphology is readily observed.

As a precedent to the utility of the bait rotifer, *L. inermis* is widely adopted by others, notably in studies of rotifer-fungal interactions (Fiałkowska and Pajdak-Stós 2018; Fiałkowska et al. 2020) and for the culture of carnivorous tardigrades (Roszkowska et al. 2021; Tůmová et al. 2022). Tardigradologists isolated *L. inermis* from the wild (Suzuki 2003) or obtained a culture from another lab (Tůmová et al. 2022). *Lecane inermis* reproduces primarily by parthenogenesis and is known to have a high reproductive capacity (Edmondson 1946; Kowalska et al. 2017). Other rotifer species with the grazing habit of feeding may be suitable to culture as bait; however, we have come to rely on *L. inermis* for its ease of culturing and high fecundity. Using a Sedgewick-Rafter counting cell, we estimated from a seven-day old, V-8 juice, *L. inermis* culture that the 3 ml added to field samples or culture dishes supplies between 6,350 and 11,000 rotifers, not counting rotifer eggs.

We speculate, on the basis of the narrow hyphal pegs, that Sparrow (1929) in his Figure 1 of *S. spinosa* may have illustrated *S. pugioniformis*. This specimen was collected from "a small pond in Fresh Pond Parkway, Cambridge, Mass." located ca. 6 km NW of the Atlantic coastal city of Boston. We collected *S. pugioniformis* from a freshwater stream 4 km inland from the Atlantic coast in Connecticut. Our collecting efforts and the highly speculative reidentification of Sparrow's Massachusetts specimen suggests the new species may be restricted to near estuarine habitats along the Atlantic coast; however, the targeted collecting effort to date is inadequate to determine with any confidence the geographic and physiographic distribution of the new species.

ACKNOWLEDGEMENTS

We thank Teodor T. Denchev and Cvetomir M. Denchev for sending publications by Arnaudov, Teodor T. Denchev for sending the photo of Dragichevsko Blato Swamp, Peter Döbbeler for formulating the Latin binomial *Sommerstorffia pugioniformis*, Sally Glockling for sharing methods of micromanipulations, Emily Kasl for samples from Florida, the late David H. Webb for a sample from Tennessee, and the following reviewers for their careful attention to the manuscript: William J. Davis, Alvin Diamond, and D. Rabern Simmons. The University of North Alabama Department of Biology provided financial support for the molecular laboratory portion of this research.

LITERATURE CITED

Arnautov, N. 1923a. Ein neuer Raderthiere (Rotatoria) fangender Pilz. Flora (Regensburg). 116:109-113

Arnautov, N. 1923b. Untersuchungen über *Sommerstorffia spinosa* nov. gen., nov. sp. Jahrbuch der Universität Sofia 19, Heft 2, Abt. 1a: 161-196.

Arnaudov, N. and N. Damova. 1947-1948. Notes on *Sommerstorffia* and *Zoophagus*. Yearbook of Sofia University Faculty of Science and Mathematics 44: 141-152.

Barron, G. L. 2004. Fungal parasites and predators of rotifers, nematodes, and other invertebrates. *In* Biodiversity of Fungi: Inventory and Monitoring Methods. G. M. Muller, G. F. Bills and M. S. Foster, eds., Elsevier Academic Press, San Diego, pp. 435-450.

Beakes G. W. and M. Thines. 2017. Hyphochytriomycota and Oomycota. *In* Handbook of the Protists, Second Edition. J. Archibald, A. Simpson and C. Slamovits, eds., Springer International Publishing, Cham, pp. 435-505.

Becking, T., A. Kiselev, V. Rossi, D. Street-Jones, F. Grandjean and E. Gaulin. 2022. Pathogenicity of animal and plant parasitic *Aphanomyces* spp and their economic impact on aquaculture and agriculture. Fungal Biology Reviews 40: 1-18.

Bryce, D. 1892. On some moss-dwelling Cathypnidae; with descriptions of five new species. Hardwicke's Science Gossip 28: 271-275.

Cejp, K. 1959. Flora CSR - Oomycetes. Nakladatelstvi Ceskoslovenské Akademie Ved, Praha.

Czeczuga, B. and D. Próba. 1980. The characteristics of the environment of *Sommerstorffia spinosa* (Oomycetes: Saprolegniales), a parasite of certain rotifers. Mycologia, 72(4): 702-707.

Czeczuga, B., B. Mazalska and M. Orłowska. 2000. New aquatic sites of the fungus *Sommerstorffia spinosa*. Acta Mycologica 35(2): 261-268.

Czeczuga, B., E. Czeczuga-Semeniuk, A. Semeniuk-Grell and J. Semeniuk. 2015. Stimulatory activity of four green freshwater sponges on aquatic mycotal communities. African Journal of Biotechnology 14(45): 3093-3100.

Dick, M. W. 2001. Straminipilous Fungi. Springer, Dordrecht.

Edmondson, W. T. 1946. Factors in the dynamics of rotifer populations. Ecological Monographs 16(4): 357-372.

Fiałkowska, E., and A. Pajdak-Stós. 2018. Temperature-dependence of predator-prey dynamics in interactions between the predatory fungus *Lecophagus* sp. and its prey *L. inermis* rotifers. Microbial Ecology 75: 400-406.

Fiałkowska, E., W. Fiałkowski and A. Pajdak-Stós. 2020. The relations between predatory fungus and its rotifer preys as a noteworthy example of intraguild predation (IGP). Microbial Ecology 79: 73-83.

Glockling, S. L., A. V. Marano, O. Lilje and F. H. Gleason. 2014. 14. Zoosporic parasites of freshwater invertebrates. In Freshwater Fungi and Fungal-like Organisms. E. B. Gareth Jones, K. D. Hyde and K. L. Pang, eds., Walter de Gruyter GmbH & Co KG, pp. 305-330.

Hoang, D. T., O. Chernomor, A. von Haeseler, B. Q. Minh and L. S. Vinh. 2018. UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35: 518-522.

Johnson T. W., R. L. Seymour and D. E. Padgett. 2002. Biology and systematics of the Saprolegniaceae. University of North Carolina at Wilmington, Wilmington.

Kalyaanamoorthy, S., B. Q. Minh, T. K. Wong, A. von Haeseler and L. S. Jermiin. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 146: 587-589.

Karling, J. S. 1952. *Sommerstorffia spinosa* Arnaudow. Mycologia 44(3): 387-412.

Karling, J. S. 1966. The Chytrids of India with a Supplement of other Zoosporic Fungi. Verlag Ferdinand Berger & Sohne OHG, Horn.

Karling, J. S. 1968. Some zoosporic fungi of New Zeland. XIII. Thraustochytriaceae, Saprolegniaceae and Puthiaceae [*sic*]. Sydowia 20: 226-234.

Kowalska, E., E. Paturej and M. Zielińska. 2017. The impact of food on the growth of clones of *Lecane inermis*, a potential bulking control agent. Environment Protection Engineering 43: 49-57.

Letunic, I. and P. Bork. 2021. Interactive Tree Of Life (iTOL) v5: An online tool for phylogenetic tree display and annotation. Nucleic Acids Research 49: W293-W296.

Miller, C. E. 1965. Annotated list of aquatic phycomycetes from Mountain Lake biological station, Virginia. The Virginia Journal of Science 14: 219-228.

Molloy, D. P., S. L. Glockling, C. A. Siegfried, G. W. Beakes, T. Y. James, S. E. Mastitsky, E. Wurdak, L. Giamberini, M. J. Gaylo, and M. J. Nemeth. 2014. *Aquastella* gen. nov.: a new genus of

saprolegniaceous oomycete rotifer parasites related to *Aphanomyces*, with unique sporangial outgrowths. Fungal Biology 118(7): 544-558.

Próba, D. 1980. Appearance of *Sommerstorffia spinosa* Arnaudov in Poland. Bulletin de l'Académie Polonaise des Sciences. Classe II. Série des sciences biologiques 27(8): 631-633.

Prowse, G. A. 1954. *Sommerstorffia spinosa* and *Zoophagus insidians* predacious on rotifers, and *Rozellopsis inflata* the endoparasite of *Zoophagus*. Transactions of the British Mycological Society 37(2): 134-150.

Riit, T., L. Tedersoo, R. Drenkhan, E. Runno-Paurson, H. Kokko, and S. Anslan. 2016. Oomycete-specific ITS primers for identification and metabarcoding. MycoKeys 14: 17-30.

Riit, T., L. Tedersoo, R. Drenkhan, E. Runno-Paurson, H. Kokko and S. Anslan. 2018. Corrigendum for:"Oomycete-specific ITS primers for identification and metabarcoding" published in MycoKeys, doi: 10.3897/mycokeys.14.9244. MycoKeys 41: 119-120.

Robideau, G. P., A. W. De Cock, M. D. Coffey, H. Voglmayr, H. Brouwer, K. Bala, D. W. Chitty, N. Désaulniers, Q. A. Eggertson, C. M. M. Gachon, C. H. Hu, F. C. Kupper, T. L. Rintoul, E. Sarhan, E. C. P. Verstappen, Y. Zhang, P. J. M. Bonants, J. B. Ristaino and C. A. Lévesque. 2011. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Molecular Ecology Resources 11(6): 1002-1011.

Rocha, S. C., C. C. Lopez-Lastra, A. V. Marano, J. I. de Souza, M. E. Rueda-Páramo and C. L. Pires-Zottarelli. 2018. New phylogenetic insights into Saprolegniales (Oomycota, Straminipila) based upon studies of specimens isolated from Brazil and Argentina. Mycological Progress 17(6): 691-700.

Roszkowska, M., D. Wojciechowska, H. Kmita, S. Cerbin, M. K. Dziuba, E. Fiałkowska, R. Sobkowiak, W. Szydło and Ł. Kaczmarek. 2021. Tips and tricks how to culture water bears: simple protocols for culturing eutardigrades (Tardigrada) under laboratory conditions. The European Zoological Journal 88(1): 449-465.

Saikawa, M. and J. Hoshino. 1986. Electron microscopy on *Sommerstorffia spinosa*, a watermold parasitic on rotifers. Mycologia 78(4): 554-561.

Saikawa, M., Y. Akamatsu, T. Sato, Y. Kawase-Izumi and M. Kitakuma. 2021. Morphological Studies on *Sommerstorffia spinosa* Parasitic on Rotifers. Bulletin of Tokyo Gakugei University, Division of Natural Sciences 73: 139-154.

Schram, M. D. and P. G. Davison. 2012. Irwin loops—a history and method of constructing homemade loops. Transactions of the Kansas Academy of Science 115: 35-40.

Scott, W. W. 1961. A monograph of the genus *Aphanomyces*. Technical Bulletin. Virginia Agricultural Experiment Station, 151, 1-95.

Segars, H. 1994-95. Zoogeography of littoral Rotifera, with special reference to the Lecanidae, Part II: Morphology and Taxonomy of *Lecane*. Doctor of Science Dissertation, Ghent University, Belgium. https://core.ac.uk/download/pdf/35106242.pdf

Sparrow Jr, F. K. 1929. A note on the occurrence of two rotifer-capturing Phycomycetes. Mycologia 21(2): 90-96.

Sparrow Jr, F. K. 1933. Inoperculate chytridiaceous organisms collected in the vicinity of Ithaca, NY, with notes on other aquatic fungi. Mycologia 25(6): 513-535.

Sparrow Jr, F. K. 1952. A contribution to our knowledge of the Phycomycetes of Cuba. III. Revista de la Sociedad Cubana de Botánica 9(4): 104-108.

Suzuki, A. C. 2003. Life history of *Milnesium tardigradum* Doyère (Tardigrada) under a rearing environment. Zoological Science 20(1): 49-57.

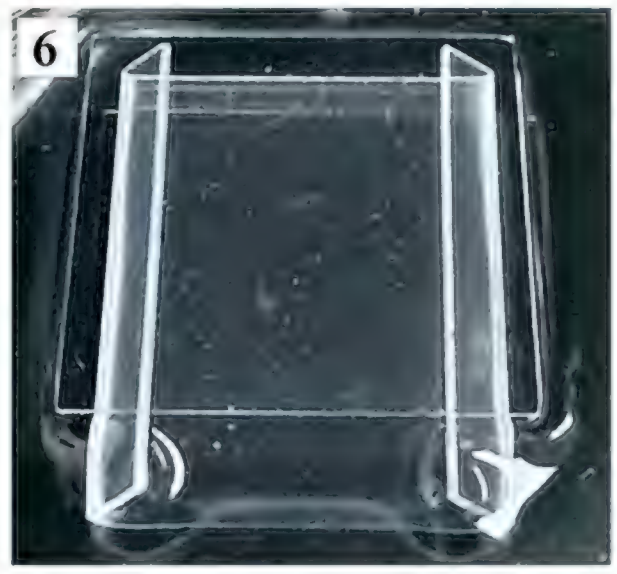
Trifinopoulos J., L. T. Nguyen, A. von Haeseler and B. Q. Minh. 2016. W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44: W232-W235.

Tůmová, M., D. Stec, L. Michalczyk and M. Devetter. 2022. Buccal tube dimensions and prey preferences in predatory tardigrades. Applied Soil Ecology 170: 104303.

White, T. J., T. D. Bruns, S. B. Lee and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR Protocols: A Guide to Methods and Applications.

M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White, eds., Academic Press, New York, pp. 315-322.

Wu, Q., B. M. Thiers and D. H. Pfister. 2004. Preparation, preservation and use of fungal specimens in herbaria. *In* Biodiversity of Fungi: Inventory and Monitoring Methods. G. M. Muller, G. F. Bills and M. S. Foster, eds., Elsevier Academic Press, San Diego, pp. 23-36.



Figures 1-6. Materials and methods. 1, arrangement of opaque material in baited dish; 2, in situ observation of floating coverslip in surface scum including duckweeds; 3-4, mount of standing pool culture—the glass pedestal prevents water at the edge of the coverslip from being pulled under the coverslip by capillary forces; 5, sitting drop culture on plastic coverslip; 6, glass coverslip held in surface water by a submerged plastic bracket (folded fragment of a Rinzl plastic slide). With proper adjustment of the water's depth, the adhesive force of water pulls the coverslip firmly against the rails of the bracket and adheres the bracket to the dish bottom.

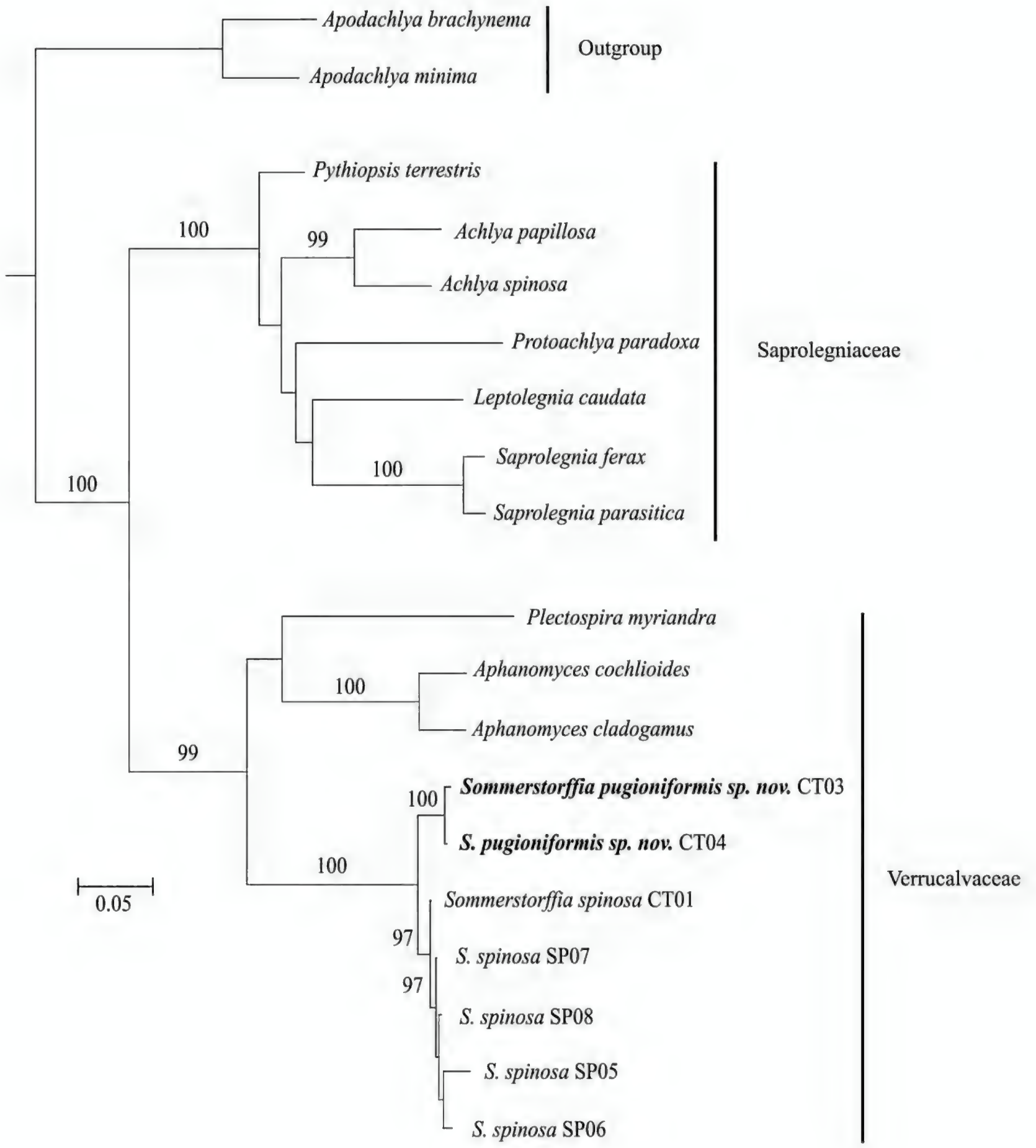
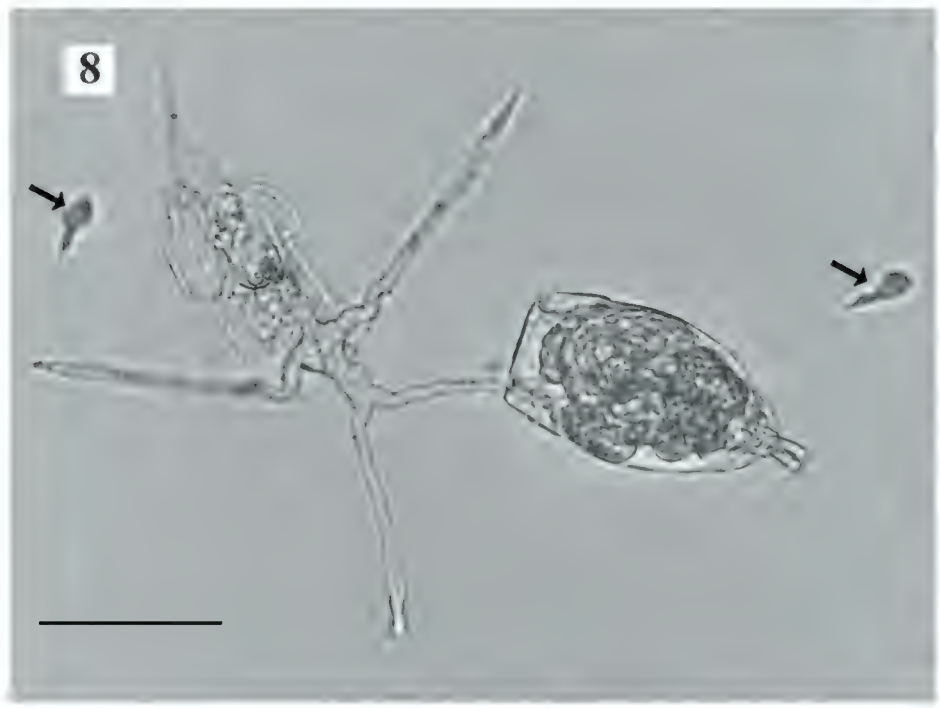
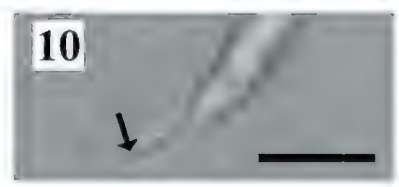
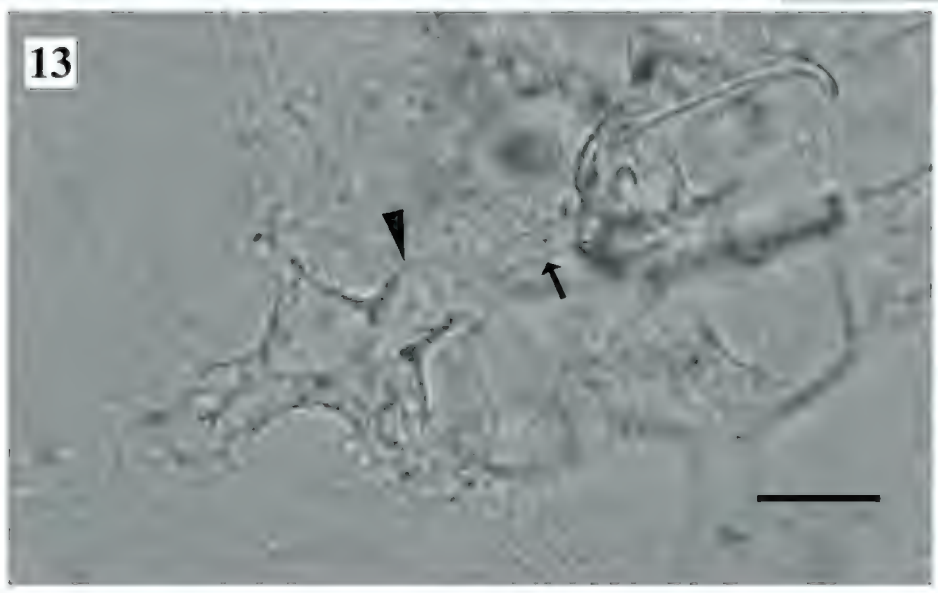


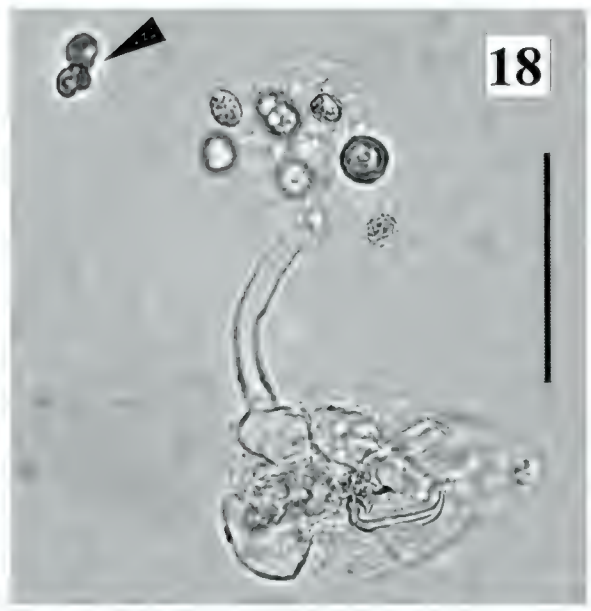
Figure 7. Maximum Likelihood phylogeny of Oomycote species from GenBank (Robideau et al. 2011) and *Sommerstorffia* isolates generated in this study. Analysis based on a partitioned data set of DNA sequences for COI and ITS with bootstrap support values of >90% shown on basal nodes. Specimens correspond to taxa and isolates in Table 1.

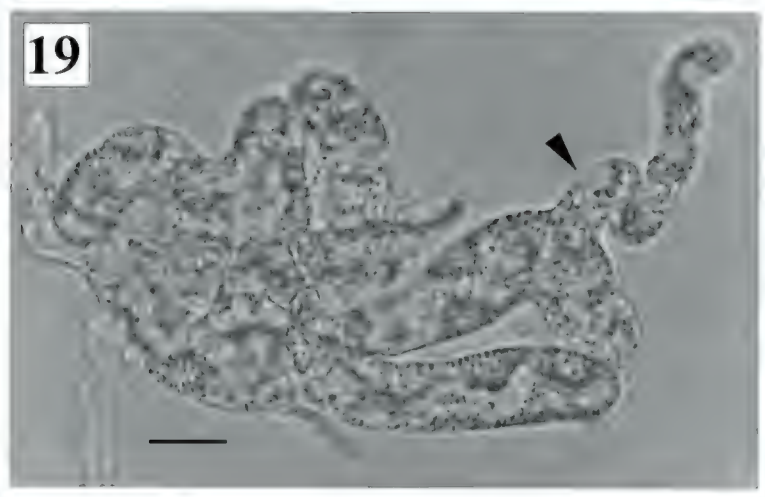


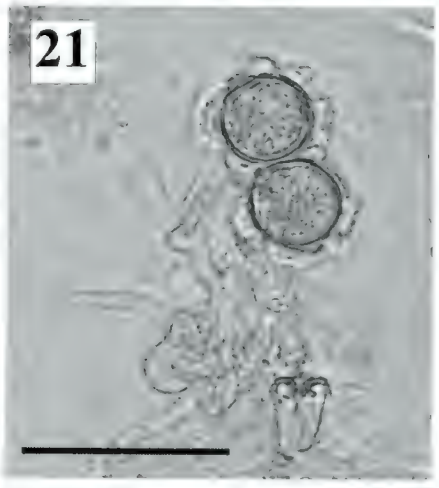




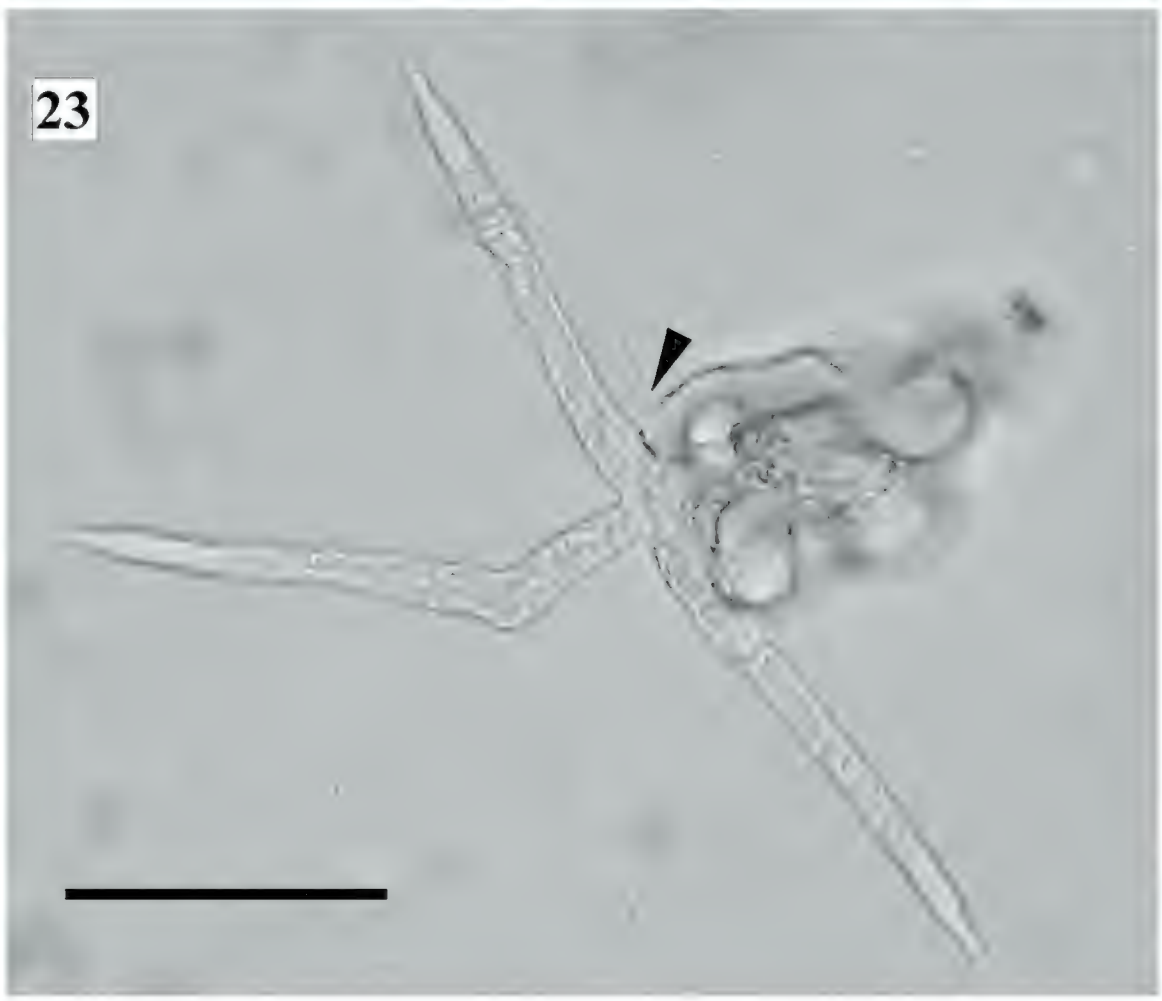
Figures 8-14. *Sommerstorffia pugioniformis*. 8, 10, 12, 13, strain CT03; 9, 11, 14, strain CT04. 8, external thallus developed from a sporeling that captured the rotifer at left, consisting of four hyphal branches, one branch with a captured rotifer; mature sporelings at arrows; 9, a three-branched thallus developed from a sporeling-captured rotifer; 10, hyphal peg with extension of hyaline material at arrow; 11, thallus with multiple branches and ghostly remains of several trapped rotifers; 12, terminal pegs at end of branches that sweep away from substrate; 13, three-branched external thallus developed from basal part of sporeling at arrowhead, the neck of sporeling (at arrow) ends in the jaws of rotifer; 14 three-branched external thallus developed from a sporeling-captured rotifer, base of capturing sporeling at arrowhead. Scale bar = 50 µm 8, 9, 11, 12, 14; 10 µm 10, 13.

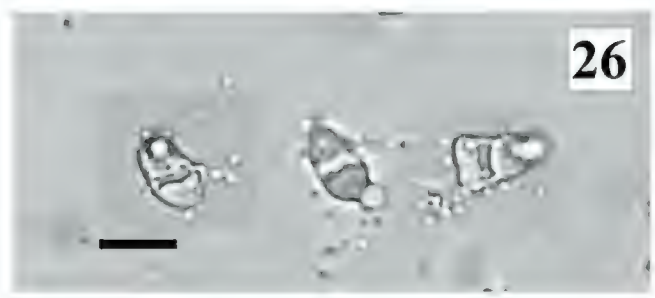




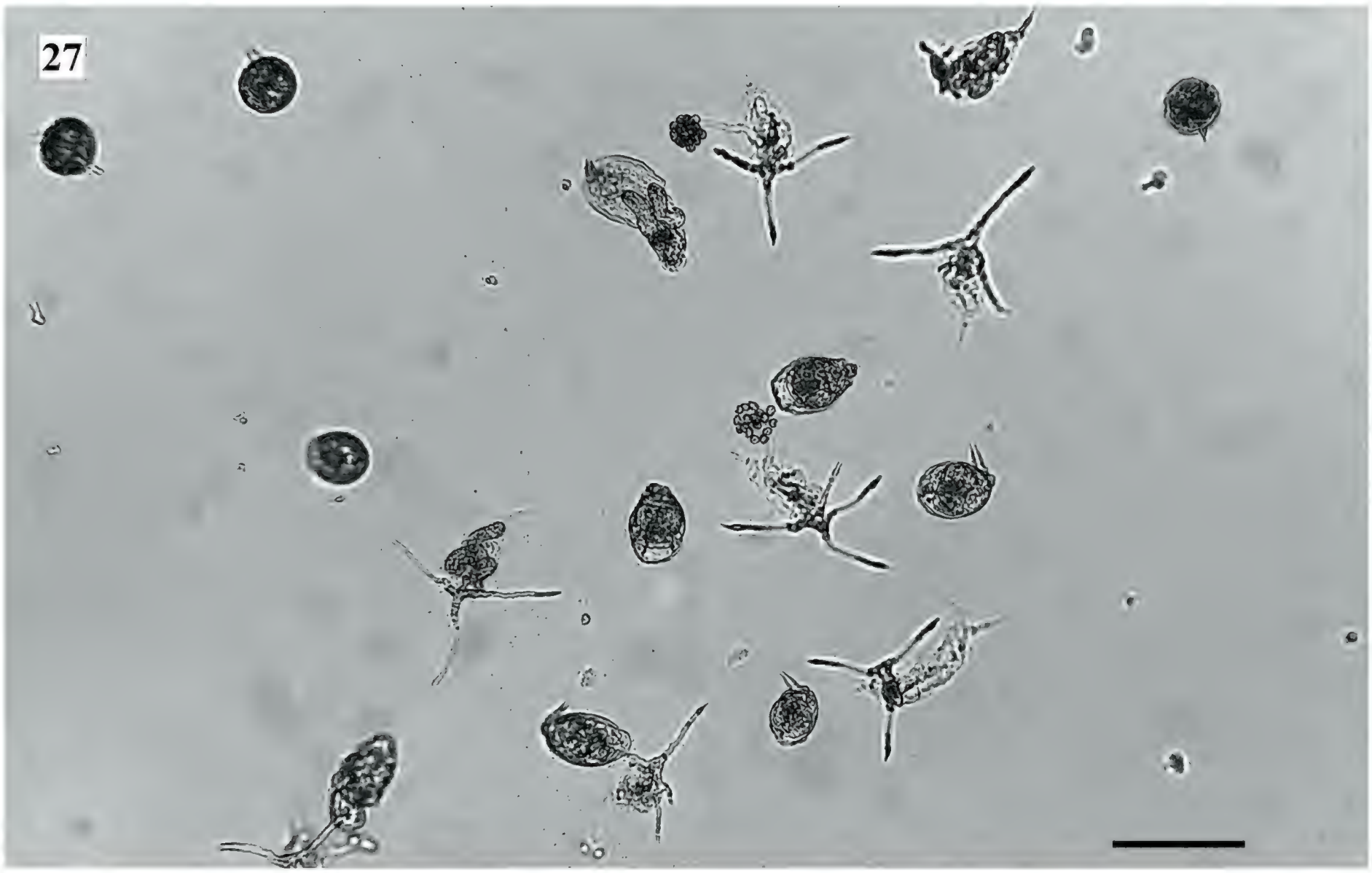


Figures 15-21. *Sommerstorffia pugioniformis*. 16, 17, 19, 21, strain CT03; 15, 18, 20, CT04. 15, mature sporeling with swollen base and slender neck, a rotifer bites the tapered end of the neck that bears a minute, swollen tip and becomes trapped; 16, three sporelings in two focal planes, at left focused on clear band in cytoplasm of the swollen base, at right focused on the necks of the sporelings and empty cyst walls at arrowheads; 17, mature sporelings with adjacent hyaline cyst walls, necks bent where joining the base by coverslip pressure; 18, endobiotic development lacking external thallus except for the exit tube leading from a lobe of the vacated zoosporangium, zoospores at arrowhead actively departing from the cystospore cluster atop the exit tube; 19, lobed zoosporangium yet to form zoospores, formation of exit tube at arrowhead; 20, oogonium on long hypha of older thallus; 21, two oogonia on shorter hyphae hidden from view. Scale bar = 10 µm 15, 16, 17, 19; 50 µm 18, 20, 21.

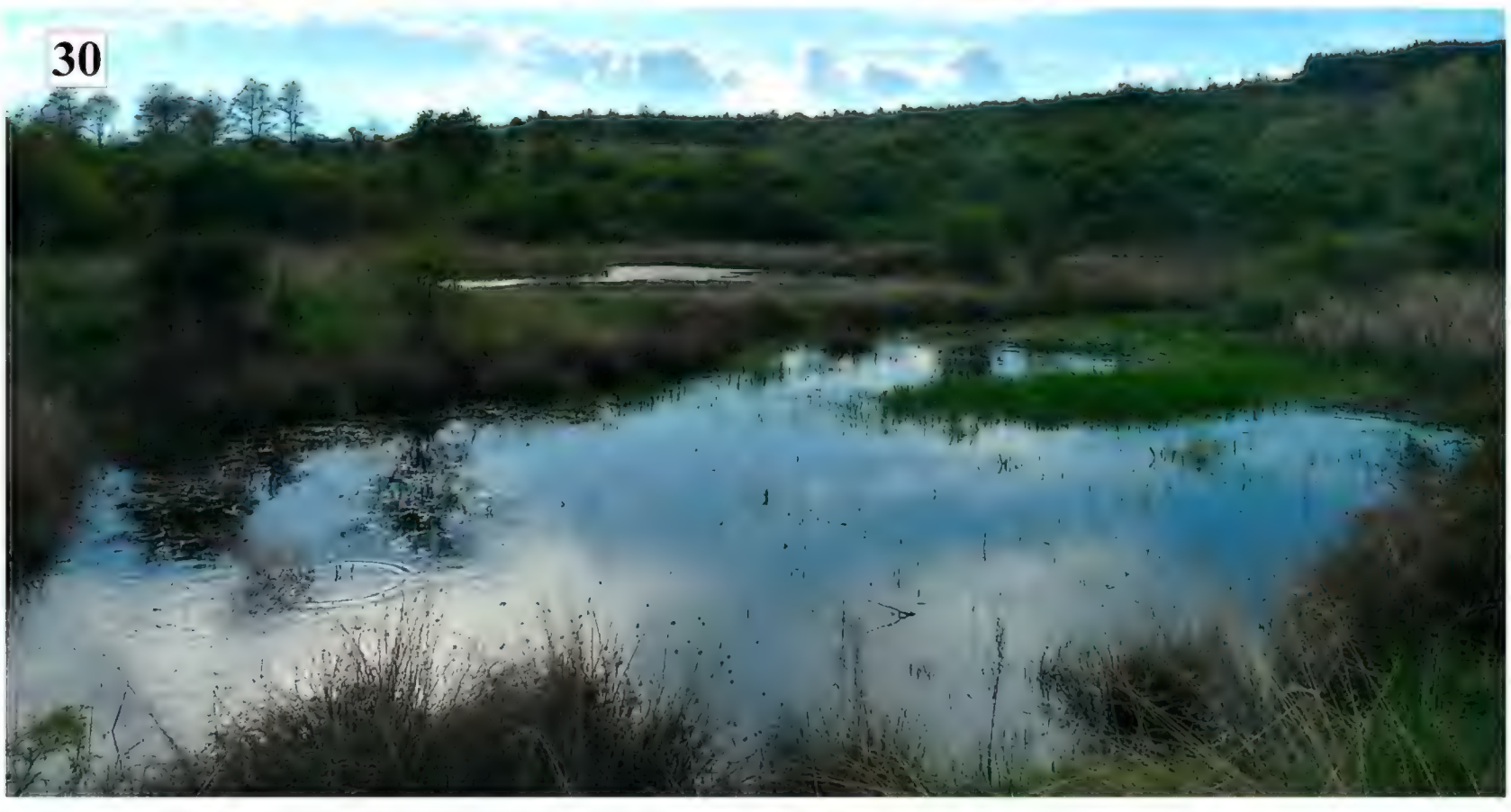




Figures 22-26. *Sommerstorffia spinosa*. Strain CT01. 22, external thallus of two branches ending in mature pegs with curled hyaline extensions, thallus developed from a sporeling that captured the rotifer now partially decomposed, cluster of cystospores atop exit tube from posterior end of rotifer; 23, external thallus of three branches ending in mature pegs, thallus developed from sporeling-captured rotifer whose body is filled with a vacated zoosporangium, exit tube (arrowhead) departing zoosporangium at anterior end of rotifer; 24, mature peg at tip of hypha bearing hyaline extension at arrow; 25-26, mature sporelings with short, nontapered necks, cyst wall at arrows. Scale bar = 50 µm 22-23, 10 µm 24-26.



Figures 27-28. *Sommerstorffia* developing under glass coverslips. 27, *S. pugioniformis* strain CT04; 28 *S. spinosa* strain CT01. Scale bar = 50 µm.



Figures 29-30. Type localities. 29, type locality of *Sommerstorffia pugioniformis*, Mill River, Connecticut, June 6, 2020; 30, type locality of *Sommerstorffia spinosa*, Dragichevsko Blato Swamp in Mt. Lyulin, Bulgaria, May 9, 2014 (photo taken by Teodor T. Denchev).

Herbivore effects on growth and survival of juvenile Damianita plants (*Chrysactina mexicana,* Compositae / Asteraceae)

O. W. Van Auken
College of Science, Department of Integrative Biology, University of Texas at San Antonio,
One UTSA Circle, San Antonio, TX 78249
oscar.vanauken@utsa.edu

C. D. Terrell
24165 IH-10 W, Suite 217, Box 712, San Antonio, TX 78257

ABSTRACT

Damianita (*Chrysactina mexicana,* Compositae/Asteraceae) is a low-density, low-growing perennial with limited presence in central Texas. Factors that control its ability to survive and growth are not known. Biological factors could be competition, herbivory or a lack of either or both. The importance of large animal herbivory on the survival and growth of juvenile plants of this species was examined in this study. Plants were protected (caged) or not protected from large animal herbivory and survival of all plants was 100%. Basal diameter, number of stems/plant and total number of leaves were not significantly different by treatment at the end of the study (*t*-tests, $P>0.05$ for all). There was no visible herbivory on any of the aboveground plant parts by the end of the study although dry mass of non-protected plants was significantly lower. Mean aboveground dry mass was significantly different for protected plants versus non-protected plants. Total dry mass was significantly different at 2.33 ± 0.31 g (not-protected, mean ± Standard error) and 3.22 ± 0.91 g (protected) (*t*-test, $P<0.05$). High density of large herbivores (white-tailed deer *Odocoileus virginianus*) was examined and caused lower dry mass of non-protected plants. Non-protected plants did not appear damaged by herbivory. However, examination of non-protected plants demonstrated selective herbivory and a reduction of 41% in stems > 5 cm in length. Herbivory on new growth in the non-protected plant treatment by *O. virginianus* reduced new stems by 41%. *Published online* ***www.phytologia.org*** *Phytologia 106(4): 68-77 (December 20, 2024). ISSN 030319430.*

KEY WORDS: herbivory, caged plants, non-caged plants, protected plants, non-protected plants, recruitment, lack of recruitment, central Texas, Edwards Plateau

---

The factors that control the growth of individual plants as well as populations and population recruitment or loss can be biotic, abiotic or mixed (Barbour et al. 1999; Maron and Crone. 2006; Mithofer and Boland 2012; Keddy 2017; Begon and Townsend 2021). This is true for common as well as rare species (Falk et al. 1996; Poole et al. 2007; Nelson-Dickinson and Van Auken 2016; Van Auken and Taylor 2020). Limiting factors could be water availability (time of rainfall and amount), other soil resources, light levels, plant competition, herbivory or some combination of factors (Ripple et al. 2024). Characteristics of a species determine how the species will compete for resources and interact with herbivores. Effects can be positive, negative, or even mixed. The growth and dry mass of Damianita (*Chrysactina mexicana,* Compositae/Asteraceae) was measured in this experiment with and without herbivory.

Chrysactina mexicana appears to be a sun species not a shade species (Correll and Johnston 1979; Powell 1998; Nokes 2001; USDA NCRS 2017). It is usually found on rocky limestone outcrops, steep slopes or cliff faces, with little soil, without trees or shade and few other plants. It is reported in central and west Texas, New Mexico and central Mexico (USDA 2017). In Mexico it is fairly widely distributed in dry regions of the Chihuahua Desert, Sierra Madre Oriental and the Trans-Mexican Volcanic Belt (Magallán-Hernández et al. 2023). Extracts from its leaves, stems, and flowers have been used to treat a number of ailments and many of its biochemicals have been identified (Cardenas-Ortega et al. 2011; Magallán-

Hernández et al. 2023). It is not reported below woodland canopies but found in high light environments not with other high light species (Wayne and Van Auken 2012; Boeck and Van Auken 2017).

The above factors do not explain why this species is relatively rare in habitats of the south-western United States. *Streptanthus bracteatus* (bracted twistflower) is a rare endemic mustard and when protected from herbivory in sun or shade it grows very well (Leonard and Van Auken 2013). Boerne bean *Phaseolus texensis* (Fabaceae = Leguminosae) is a rare perennial vine with limited distribution in central Texas woodlands. However, all Boerne bean plants observed were in *Juniperus* thickets, or very rough, steep terrain associated with *Juniperus* thickets (Nelson-Dickinson and Van Auken 2016; Van Auken and Taylor 2020). There are a lot of unconfirmed reports suggesting that large herbivores could be an important population-modifying factor controlling *C. mexicana* density, although evidence is marginal.

Populations of *Odocoileus virginianus* (white-tailed deer), and other large herbivores, have increased dramatically over most of their ranges in the past 60–100 years (Bradshaw and Waller 2016; Beschta et al. 2016). Populations of the major tree species in these communities have decreased in density (Beschta et al. 2016; Petersson et al. 2019). The same seems to be true in woodlands of central Texas and southwestern North America (Van Auken et al. 2023). Similar results for herbaceous plants have been reported (Leonard and Van Auken 2013; Bradshaw and Waller 2016; Begon and Townsend 2021).

We examined effects of large animal herbivory on the survival, growth, dry mass, and mortality of *Chrysactina mexicana*, a native perennial evergreen plant found in some native open (high light) or possibly disturbed plant communities in central Texas.

MATERIALS AND METHODS

An electronic search using Google® with the scientific name as the search item (*Chrysactina mexicana,* Damianita) turned up a number of references that were similar to what is presented in Enquist (1987). In the Spanish Language literature, a large number of studies were discovered (see Magallán-Hernández et al. 2023). Many studies specified where the species could be found geographically, pharmacological information and various items that could be purchased. Also, many chemical compounds found in extracts of tissue of this species were identified (Cardenas-Ortega et al. 2011; Magallán-Hernández et al. 2023). However, ecological or herbivory information was limited and mostly anecdotal.

Damianita (*Chrysactina mexicana,* Fig. 1) is reported from the Edwards Plateau physiographic region of central Texas, especially on some of the limestone hills, steep slopes or cliffs and ridges with little soil, without trees or shade and few other plants (Correll and Johnston 1979; Nokes 2001). It is also reported through west Texas, New Mexico and central Mexico (Elliott et al. 2014; Magallán-Hernández et al. 2023). It appears to be a sun species not a shade species (Van Auken 2018; Taiz et al. 2023).

Figure 1. Damianita plant (*Chrysactina mexicana*) in the hill country of central Texas. Photo by J. A. Marcus and copied from the Lady Bird Johnson Wildflower Center. It is a relatively low-density perennial plant with apparent limited distribution.

This study was carried out at an elevation of 428 m above mean sea level. Light levels in the open on a clear sunny day at noon were 1747 ± 29 µmol/m^2/s (mean ± SE) and in the anti-herbivory cage light levels were 1714 ± 98 µmol/m^2/s and not significantly different (*t*-test, $P = 0.9112$). Light levels were measured with a portable LICOR LI-190SA Quantum Sensor connected to a LI1000 datalogger. The soil was stony or rocky, but very shallow, calcareous, silty clay Mollisol over limestone bedrock (USDA NRCS 2017). Mean annual temperature is 18.3° C, ranging from 0.78° C in January to 34.18° C in August with high variability. Mean annual precipitation is 72.4 cm/year, highly variable, with peaks in May and September and little or none in July and August (NOAA 2018).

Plants were started with seed collected from native plants. Seeds were collected from plants growing along cliffs or bluffs on the edge of *Juniperus/Quercus* woodlands in Kendall County, Texas, USA in March (2024). Seeds were dried for seven days then spread on a potting soil mix three cm deep in a tray and covered with a very light dusting of the potting soil mix and thoroughly wetted with tap water. Seeds germinated over the next 7-14 days and 36 were carefully transplanted to 4X4 cm plastic pots. After another 30 days the plants were transplanted to 12 cm diameter plastic pots containing a 1:1 mix of Patrick series soil and sand (one/pot). Plants were kept at field capacity with tap water until the end of the experiment in August 2024.

Soil analysis indicated 7.5 g/kg carbon, 5.8 g/kg calcium, 0.65 g/kg magnesium, 0.5 g/kg total nitrogen, 6.0 mg/kg phosphorus, 69.0 mg/kg potassium, and 98.0 mg/kg sulfur (values are one-half the actual soil level because of mixture with one-half sand). The nutrient supplemented per pot was 0.1 g N (5 g/m^2) as NH_4NO_3, 0.08 g P (3.73 g/m^2) as Na_2HPO_4, 0.05 g K (2.5 g/m^2) as KCl, and 0.02 g S (1 g/m^2) as $MgSO_4$ (Bush and Van Auken 2010).

The study was completed in an open (full sun) area surrounded by *Juniperus/Quercus* woodland. The plants were at the study site in the field from July 27 until Aug. 31, 2024. All leaves greater than 5 mm in length at the end of the experiment were counted. Basal diameter and height were measured at the beginning and end of the experiment and compared by treatment. Survival and mortality were determined and then plants were harvested by clipping at ground level. The number of leaves were counted, each branch/stem was counted and length was measured and all lengths were summed to get total stem length/plant, then plant tissue was placed in paper bags for drying at 110°C for 72 hours. After that aboveground dry mass was determined. Soil and roots (belowground plant parts) were removed from the pots and placed on a sieve. Next, the soil was gently removed using a stream of tap-water. Belowground parts were removed from the sieve and placed in a paper bag then placed in an oven at 110°C for 72 hours then dry mass was determined.

There were 6 plants in each of two treatments for a total of 12 treatment plants. Treatments were non-protected (no cage) and protected (caged) (Fig. 2). Non-protected plants were placed (in pots) on the soil surface between other plants growing in the study area. Protected plants (in pots) were also placed on the soil surface in the same area, adjacent to the non-protected plants but in cages. The cages were constructed with galvanized hardware cloth with 1.3 x 1.3 cm openings and fastened with steel wire. Cages were 14.5 cm in diameter and 32 cm in height (5.7 in. diameter 12.6 in. height.).

Figure 2. Left a juvenile *Chrysactina mexicana* plant grown from seed approximately five months old. Grown in a 1:1 mix of Patrick soil and sand. The plant is approximately 14 cm tall. Right, same age plant caged to protect it from herbivory.

Mean values and standard errors were determined for measurements. Parametric statistical requirements were met and used, but variances were high. A one-way analysis of variance (ANOVA) or *t*-tests were used to determine if there were differences between treatments (Sall et al. 2001). An alpha value of 0.05 was used throughout.

RESULTS

Survival of protected and nonprotected *Chrysactina mexicana* plants was 100% at the end of the study. Herbivores (Fig. 3), mature female and juvenile white-tailed deer (*Odocoileus virginianus)* were observed selectively eating the *C. mexicana* plants the first night of the study. Later in the study both males and females were observed selectively eating plants but are not included is the figure. It was difficult to see that clipping of plant stems or browsing by the herbivores was occurring.

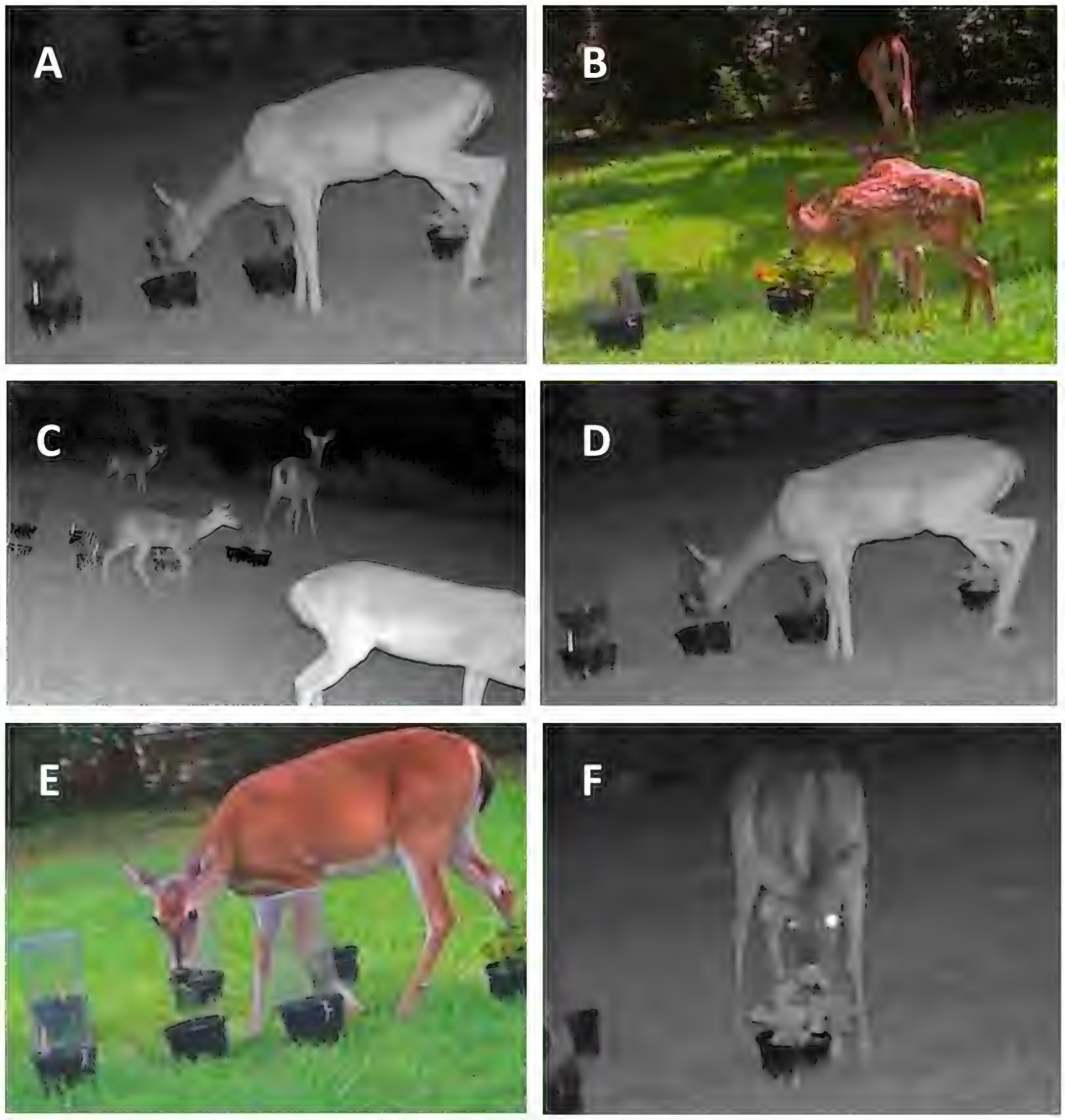


Figure 3. Top (A), female white-tailed deer sniffing/examining and eating (*Chrysactina mexicana*). potted plant placed in the study area. (B), faun eating a potted geranium plant. A number of white-tailed deer (C) in the study area ignoring the study plants. (D and E), female white-tailed deer sniffing/eating plants. (F), a female white-tailed deer eating other potted plants placed in the study area not Damianita plants (photos by C. D. Terrell).

Height of non-caged plants (Fig. 4) increased from 13.3 ± 2.1 cm (mean ± se) at the start of the experiment to 19.0 ± 1.7 cm at the end of the experiment (August 31, 2024-six weeks). Caged plants increased in height from 14.3 ± 2.5 cm at the start to 19.7 ± 3.4 cm at the end of the experiment. There was no significant difference in plant height for the caged versus non-caged plants at the end of the experiment (*F*-test, *P*= 0.370). At the beginning and the end of the study, basal diameter (Fig. 5 A) was not significantly different between protected plants and non-protected plants (beginning 2.16 ± 0.36 mm protected compared with 2.01 ± 0.50 mm non-protected; at the end values were 3.97 ± 0.42 mm protected and 2.79 ± 0.33 mm non-protected, t-test, $P > 0.05$ for both; Fig. 5 A). At the end of the study there was not a significant difference in the number of stems or branches in the non-caged (18 ± 5/plant) versus the caged treatments (24 ± 7/plant) (*t*-test, *P* = 0.1095, Fig. 5 B). Number of total leaves on *C. mexicana* plants counted at the end of the herbivory experiment were not significantly different between the non-protected (361 ± 72) versus the protected plants (530 ± 208) (*t*-test, *P*= 0.09, Fig. 5 C). At the end of the experiment the total (summed) stem or branch length for the non-protected plants was 77 ± 24 cm and 122 ± 72 cm for nonprotected plants and they were significantly different (*t*-test, *P* = 0.036; Fig. 5 D). There were no flowers produced in either treatment.

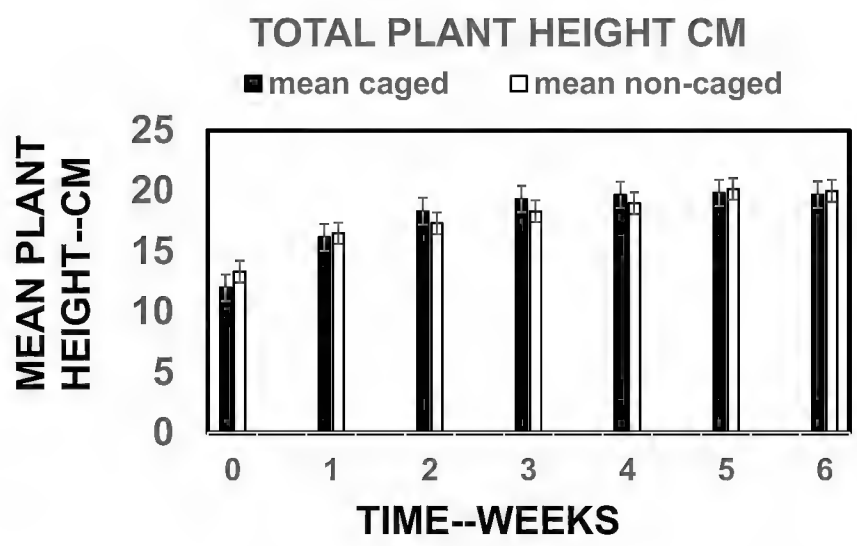


Figure 4. *Chrysactina mexicana* overall plant height measured in cm weekly from the beginning of the experiment until the end of the experiment (August 31, 2024). There were no significant differences between the caged or non-caged plants (protected vs. non-protected, *F*-test, $P > 0.05$).

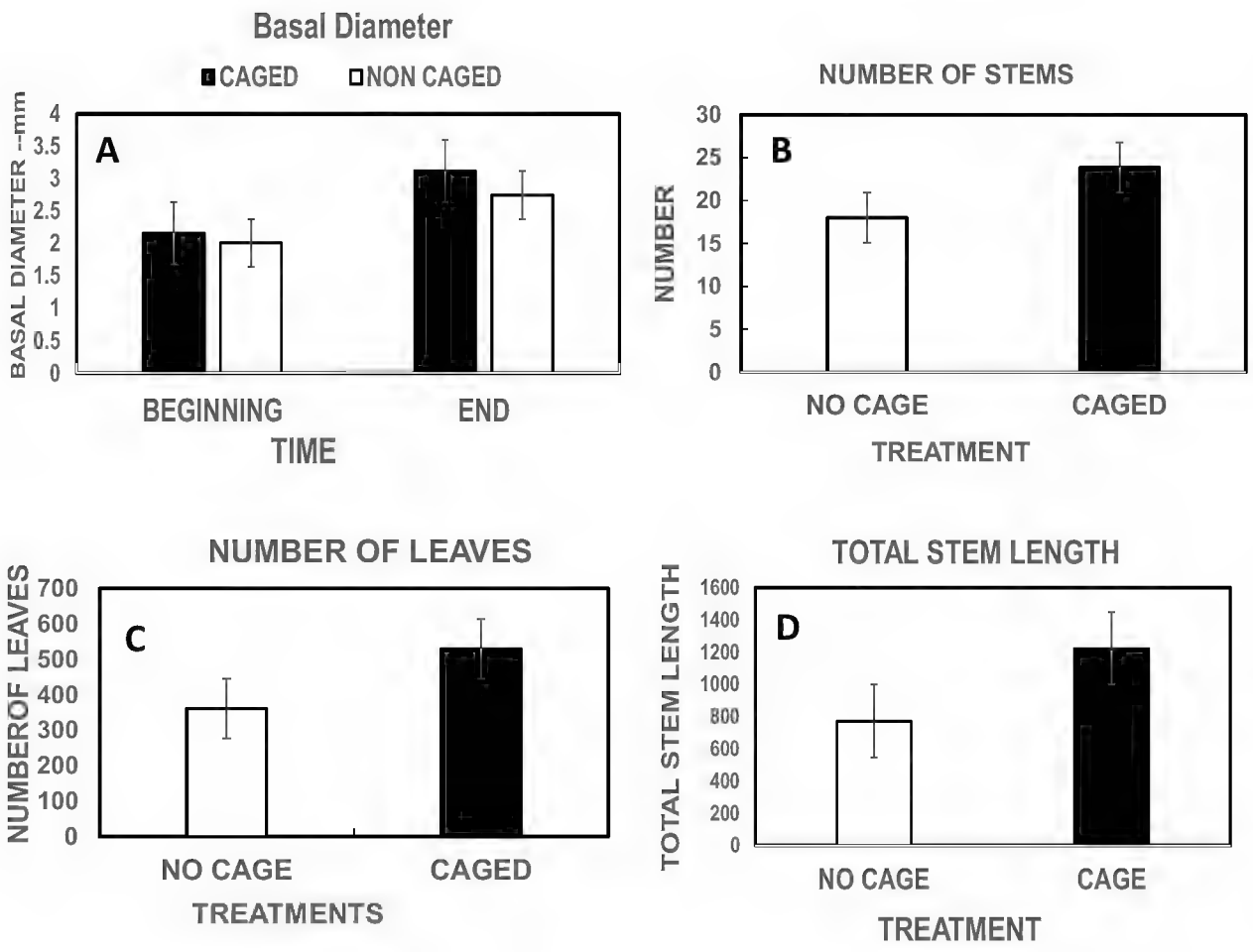


Figure 5. (A) Basal diameter (mm) of *Chrysactina mexicana* plants measured before and at the end of the herbivory experiment. Beginning and end treatments were not significantly different (NSD) (*t*-tests, $P>0.05$ for both) but the same treatments at the end were significantly different (SD) (*t*-tests, $P<0.05$). (B) Number of stems at the end of the experiment were NSD (*t*-test, *P*=0.1095). (C) Number of leaves at the end of the experiment were NSD (*t*-test, *P*=0.0895). (D) Total stem length at the end of the experiment was SD (*t*-test, *P*=0.0357). Plants were protected or non-protected.

Also, at the end of the experiment there was no significant difference between the number of small stems (0-5 cm height) in the protected plants and the non-protected plants (Fig. 6). However, for all of the stems (branches) 6 cm in height or greater, there were 41% fewer in the non-protected treatment (Fig. 6, 57 versus 96 stems).

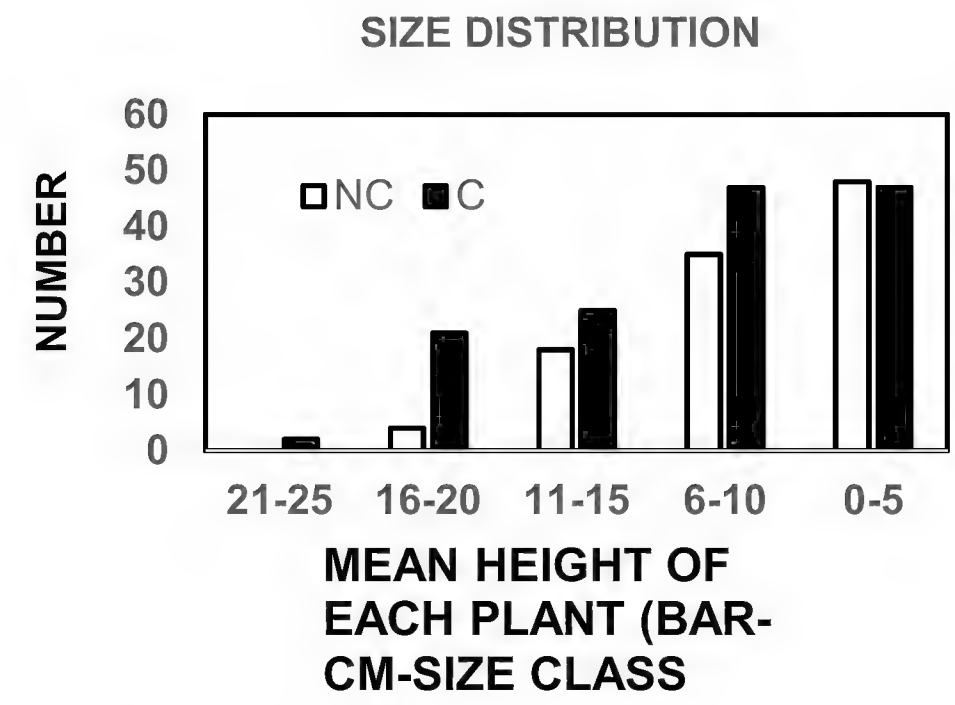


Figure 6. Number of branches in 5 cm length segments for each treatment at the end of the experiment. Height was divided into 5 cm segments and measured for each plant branch in each treatment (caged or not caged) and then totaled for each treatment. The number of stems in the two treatments was not significantly different at the end of the experiment (*t*-test, $P > 0.05$). However, when differences between treatments were compared by size, the number of branches on the protected plants (caged) was significantly different than the non-protected plants (no cage) (*F*-test, $P = 0.0018$).

This is also observed in the dry mass measurements (Fig. 7). Mean belowground dry mass of protected plants was 0.74 ± 0.38 g (mean ± SE) and not significantly different from non-protected plants at 0.68 ± 0.13 g (*t*-test, $P = 0.78$, Fig. 7). But, mean aboveground dry mass of protected plants was 2.46 ± 0.78. g and significantly different from non-protected plants at 1.65 ± 0.26 g (*t*-test, $P = 0.048$, Fig. 7) and mean total dry mass of protected plants was 3.20 ± 0.91 g and significantly different from non-protected plants at 2.33 ± 0.31 g (*t*-test, $P = 0.028$).

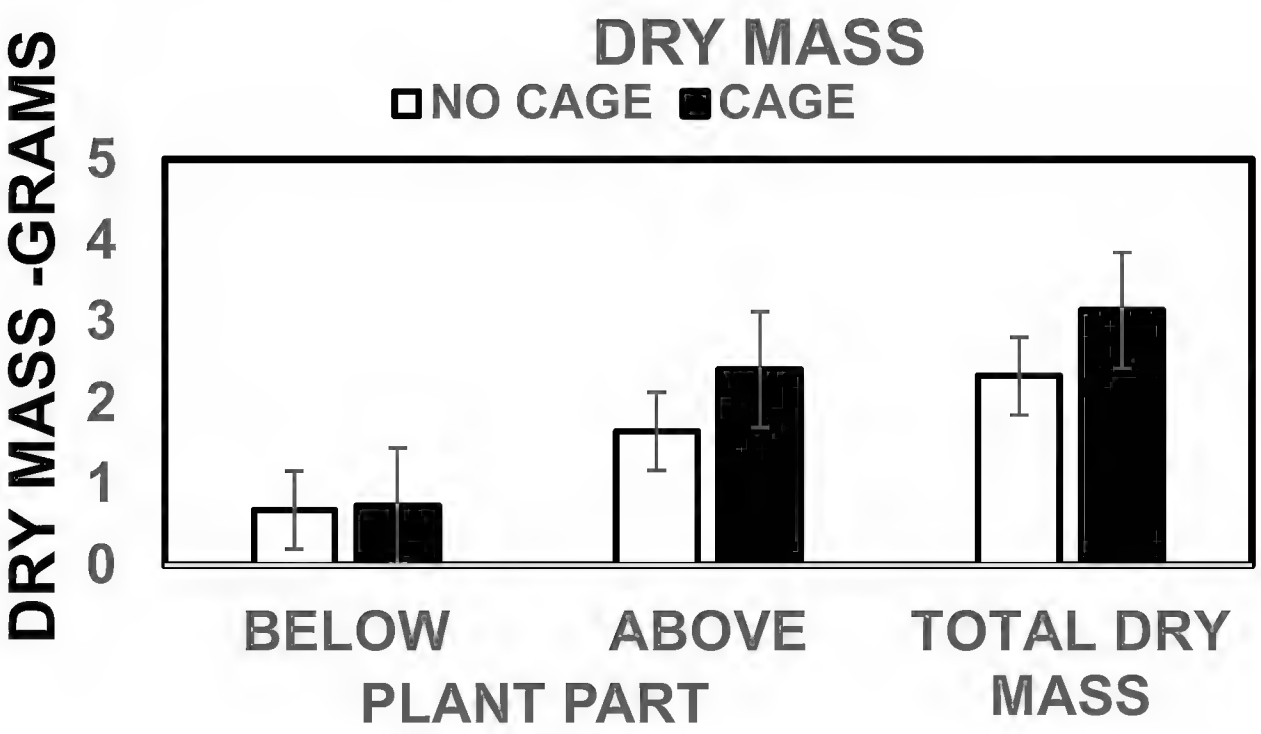


Figure 7. Below ground, aboveground and total dry mass of *Chrysactina mexicana* plants measured at the end of the experiment with or without a cage. There was NSD on below ground dry mass (*t*-test, $P > 0.05$). However, aboveground and total dry mass treatments were SD at the end of the experiment (*t*-tests, P=0.04808 and 0.0042 respectively).

DISCUSSION

Lack of recruitment of woody and herbaceous plants has been reported from all over the world including central and western Texas with juveniles of many species not entering the adult population (Van Auken et al.2017; Van Auken and Taylor 2020; Begon and Townsend 2021; Van Auken et al. 2023; Ripple et al. 2024). The main reason for lack of recruitment seems to be large animal herbivory (Abrams 2003; Nelson-Dickinson and Van Auken 2016; Van Auken and Taylor 2020; Van Auken et al. 2023). Evidence

has shown recruitment decline of woodland and forest plant species throughout North America because of herbivory by large populations of cervids particularly *Odocoileus virginianus* (white-tailed deer) and Cervus canadensis (elk) (Lorimer 1992; Kain et al. 2011; Bradshaw and Waller 2016; Ripple et al. 2024). Other reasons including lack of fire or drought seem secondary (Collins and Wallace 1990; Barbour et al. 1999; Keddy 2017; Begon and Townsend 2021).

Density of *O. virginianus* in central Texas (1 deer/5 ha) is higher than anywhere else in North America (Doughty 1983; Abrams and Ross 2015; Fulbright and Ortega-S. 2008; Makar 2017). In areas with lower densities of white-tailed deer juvenile woody plants and herbaceous plant density is higher and some recruitment or replacement is occurring. Early in the last century there was a major decline in populations of large herbivores, but recovery of the populations has happened and current populations are high (Doughty 1983).

For *Chrysactina mexicana* in the current study, all plants protected or not survived over the entire study (100%). However, aboveground and total dry mass was reduced (Fig. 7) in the open or non-caged plants indicating herbivory. The herbivory was selective, that is, new growth was selected by *O. virginianus* not the mature stems which was difficult to observe. But not difficult to detect in the dry mass measurements.

In the current study, cages used were small and had small openings (1 cm^2), large enough to allow entry of small insects but this did not seem to matter concerning herbivory or biomass. There was no evidence of herbivory on the protected plants (caged, visual inspection). *Odocoileus virginianus* could get to the non-protected plants but not the protected ones, which was true for other herbivores, but that did not seem to matter. This suggests that white-tailed deer were a potential cause of low density or limited distribution of this species as in other studies (Van Auken 1988; Russell and Fowler 2002; Leonard and Van Auken 2013; Bradshaw and Waller 2016; Van Auken 2018; Van Auken and Taylor 2020; Van Auken et al. 2023). We present pictures of white-tailed deer inspecting *C. mexicana* plants but no indication of clipping mature plant leaves or stems or eating them (Fig. 3).

The increase in height and dry mass of protected plants was noted and due to selective herbivory of the non-caged plants not to slight shading of the caged plants This suggests that the density of large herbivores is important for recruitment of juveniles of *C. mexicana* plants into the adult population. Other factors seem to be involved, such as production and presence of toxins that prevent herbivory to mature tissue, specific habitat site requirements for this species, competition with other species, or possibly of a combination of these factors (Van Auken et al. 2023).

The importance of herbivory to many if not most species in these central Texas woodlands and other parts of the world has been shown (Van Auken 1988; Russell and Fowler 2002; Leonard and Van Auken 2013; Bradshaw and Waller 2016; Van Auken et al. 2017; Petersson et al. 2019; Van Auken and Taylor 2020; Van Auken et al. 2023). In addition, climate, especially the amount and timing of rainfall or lack of rainfall, may be a cause of mortality and lack of recruitment (Johnson et al. 2018), but was not examined in this experiment and no evidence of fire was reported in the study area where this species was found as was reported for other areas (Andruk et al. 2014). Intermittent or episodic recruitment may be occurring in these *C. mexicana* populations, but this is unknown. There was digging and rooting, which suggests the presence of feral hogs, but study plants were not disturbed. In spite of demonstrated herbivory, the future distribution and population density of this species will require more widespread density studies and more specific habitat measurements.

ACKNOWLEDGEMENTS

The method for germinating the seeds is adjusted from recommendations from C. Janzow and J. Nokes. David D. Diamond, Jason Gagliardi, Mathew Grunstra, Wendy Leonard, and Kevin Eddy read an earlier copy of this manuscript and offered many helpful suggestions. Jurgen Engelberth suggested several Spanish language studies and C. Wu helped find some rare studies. All their help was greatly appreciated.

LITERATURE CITED

Abrams, M. D. 2003. Where has all the white oak gone? Bioscience 53: 927–939. doi.org/10.1641/00063568(2003)053[0927:WHATWO]2.0.CO;2.

Adams, K. P. and M. Ross. 2015. QDMA's whitetail report. Quality Deer Management Association, Bogart,Adruk Georgia.

Andruk, C. M., C. Schwope and N. L. Fowler. 2014. The joint effects of fire and herbivory on hardwood regeneration in central Texas woodlands. For. Ecol. Manag. 334: 193-200.

Barbour, M. G., J. H. Burk, W. D. Pitts, F. S. Gilliam and M. W. Schwartz. 1999. Terrestrial Plant Ecology. Addison Wesley Longman. Inc. Menlo Park, Cal.

Begon, M., and C. R. Townsend. 2021. Ecology: from individuals to ecosystems. Wiley and Company, New York.

Boeck, A., and O. W. Van Auken. 2017. Distribution and factors that influence the distribution of *Heliotropium tenellum* (Boraginaceae) in central Texas cedar glades. Tex. J. Sci. 68: 3-21.

Bradshaw, L., and D. M. Waller. 2016. Impacts of whitetailed deer on regional patterns of forest tree recruitment. For. Ecol. Manag. 375: 1–11.

Bush, J. K., and O. W. Van Auken. 2010. Competition between *Schizachyrium scoparium* and *Buchloe dactyloides*: The role of soil nutrients. J. Arid Env. 74: 49–53.

Cardenas-Ortega, N. C., B. I. Juarez-Flores, J. M. Pinos-Rodriguez and J. R. Aguirre-Rivera. 2011. Biocide effects of *Chrysactinia mexicana* A. Gray. Pages 119-134. in Plant-Phytochemicals in animal nutrition. Salem, A.-F. Z. M. Nova Science Pub. Inc., New York.

Collins, S. L., and L. L. Wallace. 1990. Fire in North American tallgrass prairies. Univ. Ok. Press, Norman, OK.

Correll, D. S., and Johnston, M. C. 1979. Manual of the Vascular Plants of Texas. Texas Research Foundation, Renner, TX.

Doughty, R. W. 1983. Wildlife and Man in Texas: Environmental Change and Conservation. Texas A&M University Press, College Station, TX.

Elliott, L. F., D. D. Diamond, C. D. True, C. F. Blodgett, D. Pursell, D. German and A. Treuer-Kuehn. 2014. Ecological mapping systems of Texas: summary report. Texas Parks and Wildlife Department, Austin, Texas. Available at: https://tpwd. texas.gov/gis/programs/landscape-ecology/supportingdocuments/final-summary-report/view. Accessed 20 January 2020.

Enquist, M. 1987. Wildflowers of the Texas Hill Country. Lone Star Botanical, Austin, TX.

Falk, D. A., C. I. Millar and M. Olwell. 1996. Restoring diversity: strategies for reintroduction of endangered plants. Island Press, Washington, DC.

Fulbright, T. E., and J. A. Ortega-S. 2008. White-tailed deer habitat: ecology and management on rangelands. Texas A&M University Press, College Station, TX.

Johnson, D. M., J.-C. Domec, Z. C. Berry, A. M. Schwantes, K. A. McCulloh, D. R. Woodruff, H. W. Polley, R. Wortemann, J. J. Swenson, D. S. Mackay, N. G. McDowell and R. B. Jackson. 2018. Co-occurring woody species have diverse strategies and mortality rates during an extreme drought. Plant Cell Env. 41: 576–588.

Kain, M., L. Battaglia, A. Royo and W. P. Carson. 2011. Overbrowsing in Pennsylvania creates a depauperate forest dominated by an understory tree: results from a 60-year-old deer exclosure. J. Torrey Bot. Soc. 138: 322–326. doi:10.3159/TORREY-D-11-00018.1.

Keddy, P. A. 2017. Plant ecology: origins, processes, consequences. Cambridge Univ. Press. NY.

Leonard, W. J. and O. W. Van Auken. 2013. Light Levels and Herbivory Partially Explain the Survival, Growth, and Niche Requirements of *Streptanthus bracteatus* A. Gray (Bracted Twistflower, Brassicaceae), a Rare Central Texas Endemic. Nat. Areas J. 33: 276-285.

Louda, S. M. and J. E. Rodman. 1996. Insect herbivory as a major factor in the shade distribution of a native crucifer (*Cardamine cordifolia* A. Gray, bittercress). J. Ecol. 84: 229–237.

Lorimer, C. G. 1992. Causes of the oak regeneration problem. Pages 14–40 in Oak regeneration: serious problems, practical recommendations (D. L. Loftis and C. E. McGee, editors). USDA Southeastern Forest Experiment Station, Knoxville, Tennessee.

Magallán-Hernández, F., J. A. Valencia-Hernández and R. Sánchez-Castillo. 2023. For the conservation and use of *Chrysactinia Mexicana* an aromatic and medicinal plant native to Mexico. Polibotanica 55: 145-159.

Makar, V. 2017. Estimating white-tailed deer abundance within Albert and Bessie Kronkosky State Natural Area. M.S. thesis, University of Texas San Antonio, Texas.

Maron, J. L. and E. Crone. 2006. Herbivory: effects on plant abundance, distribution and population growth. Proc. Royal Soc. B 273: 2575–2584.

Mithofer, A. and W. Boland. 2012. Plant defense against herbivores: chemical aspects. Ann. Rev. Plant Bio. 63: 431-450.

Nelson-Dickinson, T. L. and O. W. Van Auken. 2016. Survival, growth and recruitment of bigtooth maple (*Acer grandidentatum*) in central Texas relict communities. Nat. Areas J. 36: 174-180. https://doi.org/10.3375/043.036.0209

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION, [NOAA]. 2018. National Centers for Environmental Information. Climate Data Online. https://www.ncei. noaa.gov/cdo-web/. Retrieved July 14, 2018.

Nokes, J. 2001. How to Grow Native Plants. University of Texas Press, Austin, TX.

Petersson, L. K., P. Milberg, J. Bergstedt, J. Dahlgren, A. M. Felton, F. Gotmark, C. Salk and M. Lof. 2019. Changing land use and increasing abundance of deer cause natural regeneration failure of oaks: Six decades of landscape-scale evidence. For. Ecol. Manag. 444: 299–367.

Poole, J. M., W. R. Carr, D. M. Price and J. R. Singhurst. 2007. Rare plants of Texas. Texas A&M Nature Guides. Texas Parks and Wildlife. Everbest Printing Louisville, KY.

Powell, A. M. 1998. Trees and Shrubs of the Trans-Pecos and Adjacent Areas. University of Texas Press, Austin, TX.

Ripple, W. J., C. Wolf, R. L. Beschta, A. D. Craig, Z. S. Curcija, E. J. Lundgren, L. C. Satterfield, S. T. Woodrich and A. J. Wirsing. 2024. A shifting ecological baseline after wolf extirpation. BioScience 74: 430-434.

Russell, F. L., D. B. Zippin and N. L. Fowler. 2001. Effects of white-tailed deer (*Odocoileus virginianus*) on plants, plant populations and communities: a review. Amer. Midl. Nat. 146: 1-26.

Russell, F. L. and N. L. Fowler. 2002. Failure of adult recruitment in *Quercus buckleyi* populations on the eastern Edwards Plateau, Texas. American Midland Naturalist 148: 201–217.

Sall, J., A. Lehman and L. Creighton. 2001. JMP start statistics: a guide to statistics and data analysis using JMP and JMP IN software. Duxbury Thomson Learning, Pacific Grove, CA.

Stormer, F. A. and W. A. Bauer. 1980. Summer forage use by tame deer in northern Michigan. J. Wild. Manag. 44: 98–106.

Taiz, L., I. M. Moller, A. Murphy and E. Zeiger, 2023. Plant Physiology and Development. Sinauer Associates, NY.

USDA NRCS (United States Department of Agriculture, Natural Resources Conservation Service). 2017. Web soil surveys, soil survey staff. USDA NRCS, Washington, D.C. Available at: http://websoilsruvey.nrcs.usda.gov/app/. Accessed 10 May 2024.

Van Auken, O. W. 1988. Woody vegetation of the southeastern escarpment and plateau, pp. 43–55. In: B. B. Amos and F. R. Gehlbach, eds. Edward's Plateau Vegetation: Plant Ecological Studies in Central Texas. Baylor University Press, Waco, TX.

Van Auken, O .W., Taylor, D. L., Shen, C. and Bush, J. K. 2017. Structure of isolated *Acer grandidentatum* (bigtooth maple) Communities and Potential Population Changes. Amer. J. Plant Sci. 98: 232-240.

Van Auken, O. W. 2018. Ecology of Plant Communities of South-Central Texas. Scientific Research Publishing, Wuhan.

Van Auken, O. W. and D. L. Taylor. 2020. Survival of juvenile *Acer grandidentatum* (bigtooth maple, Aceraceae) in Central Texas Woodlands. Amer. J. Plant Sci. 11: 413-425.

Van Auken, O. W., J. K. Bush, D. L. Taylor and J. R. Singhurst. 2023. Lack of woody species recruitment in isolated deep canyon deciduous woodlands in Central Texas, USA. J. Torrey Bot. Soc. 150: 525-537. https://doi.org/10.3159/TORREY-D-23-00009.1

Wayne, E. R. and O. W. Van Auken. 2012. Comparisons of gas exchange of several sedges and C_4 grasses in associated savanna communities. Phytologia. 94: 71-90.